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Lovastatin Biosynthesis and Enzymatic Diels Alder Reactions

by

Johan Philip van den Heever

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Master of Science

Department of Chemistry

Edmonton, Alberta

Fall 2001

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Lovastatin Biosynthesis and Enzymatic Diels Alder Reactions" submitted by Johan Philip van den Heever in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

The putative Diels Alder reaction during the biosynthesis of lovastatin **1** and compactin **2** was examined using an *Aspergillus nidulans* fungal strain containing the lovastatin nonaketide synthase gene (LNKS, *lovB*), prepared by Professor Hutchinson (University of Wisconsin).

The enzymatic Diels Alder reaction was evaluated by synthesis and testing hexaketide substrate analogues with purified *lovB* enzyme. The substrates tested were (*6R*)-(*E,E,E*)-6-methyldodeca-2,8,10-trienoic acid *N*-acetylcysteamine thioester **40**, ethyl (*6R*)-(*E,E,E*)-6-methyldodeca-2,8,10-trienoate **41** and (*6R*)-(*E,E,E*)-6-methyldodeca-2,8,10-trienthioic acid *S*-ethyl ester **77**. Only compound **40** was observed to be a good substrate for *lovB* enzyme, affording a mixture of non-enzymatic cyclized products, as well as an enzymatically cyclized product corresponding to the stereochemistry of the lovastatin **1** precursor, 4a,5-dihydromonacolin L **32**.

Three compactin analogue intermediate substrates namely dodeca-(*E,E,E*)-2,8,10-trienoic acid *S*-(2-acetylamino ethyl) ester **78**, dodeca-(*E,E,E*)-2,8,10-trienoic acid ethyl ester **79** and dodeca-(*E,E,E*)-2,8,10-trienthioic acid *S*-ethyl ester **80** were also tested with purified *lovB* enzyme. None of these compounds were observed to be good substrates for *lovB*.

LovB represents the first naturally occurring Diels Alderase enzyme to be purified, and one of the few fungal PKS enzymes to be isolated and characterized.

Acknowledgements

I wish to thank Prof. John C. Vederas for accepting me as a graduate student, as well as for his patience, guidance and expertise. I would also like to thank Dr. Andrew Sutherland for his help with the enzymatic studies and for proofreading my thesis, as well as Dr. Renata Jankowski for proofreading my thesis. I would also like to express my thanks to Jim Hoyle for his help with developing the GC methodology I used. I also want to thank all the members of our research group for their help at various times.

Mostly I would like to thank my wife, Rika, for agreeing to move to Canada with me and helping me to make all of this possible.

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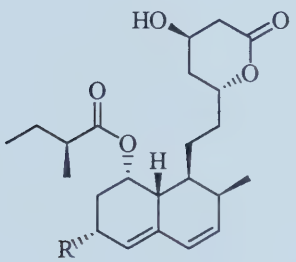
1 Introduction

The aim of these investigations is to demonstrate that a putative enzyme-catalyzed Diels-Alder reaction[1] is involved in the formation of the decalin moiety of lovastatin[2] **1** and compactin[3] **2** (Figure 1). The substrate specificity of this enzyme is also investigated.



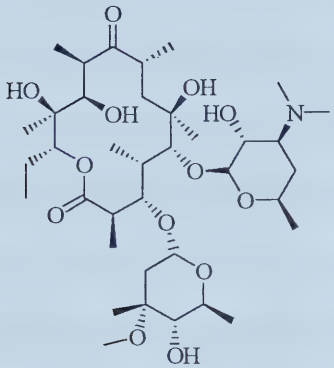
R=CH_3 lovastatin (**1**)
R=H compactin (**2**)

Figure 1. Structures of lovastatin **1** and compactin **2**.

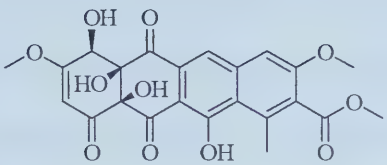
1.1 Properties and importance of polyketides

Polyketides are a large family of complex natural products that have their origin in simple carboxylic acid monomers.[4] Although the biological role of these natural products in the native producing organisms are often unclear, their useful pharmaceutical, veterinary and agrochemical properties make them indispensable tools in our fight against infectious diseases and other ailments. Some beneficial polyketides include antibiotics such as erythromycin A **3** and tetracenomycin C **4** (Figure 2), immunosuppressants (FK 506) **6** and antiparasitics like monensin A 7, as well as

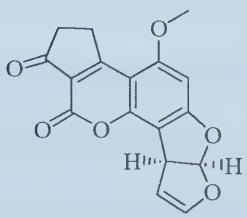
cholesterol-lowering agents like lovastatin **1** and compactin **2**. Not all polyketides are useful, as some are highly toxic, for example aflatoxin B_1 **5** (Figure 2).



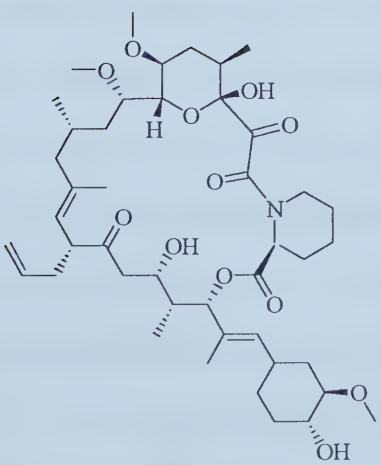
Erythromycin A **(3)**



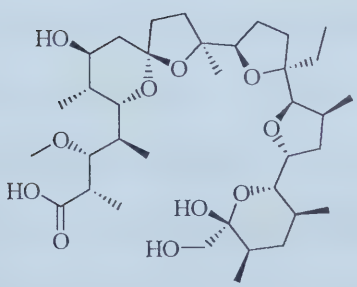
Tetracenomycin C **(4)**



Aflatoxin B_1 **(5)**



FK 506 **(6)**



Monensin A **(7)**

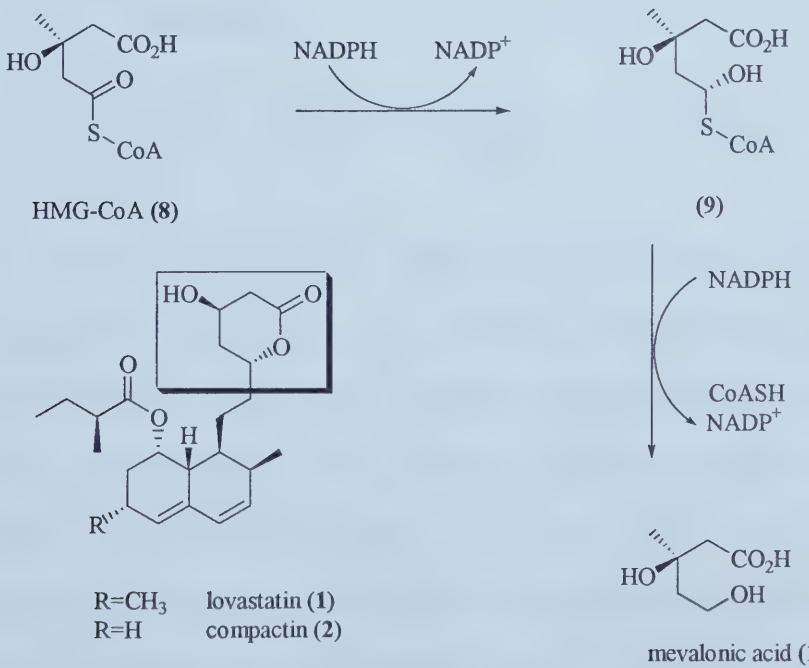
Figure 2. Some examples of polyketide secondary metabolites.

Lovastatin **1** and compactin **2**, as well as analogues of these compounds are important cholesterol-lowering compounds.[5] Cholesterol is the precursor of steroid hormones and bile acids,[6] and is a major component of cell membranes in healthy organisms, where it is essential for maintaining the cell membrane integrity. Two types of cholesterol are found in the blood, namely low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol. LDL cholesterol is secreted by the liver and delivered to body cells *via* the blood. Prolonged occurrence of excessively high concentrations of LDL cholesterol in blood leads to the accumulation of fatty deposits in the arteries (arteriosclerosis). This fatty buildup in the arteries increases the risk of developing cardiovascular diseases like thrombosis and strokes. Controlling the levels of LDL cholesterol in the blood is therefore vital in reducing the occurrence of several cardiovascular diseases in humans. A higher ratio of HDL cholesterol to LDL cholesterol in blood is desirable, since HDL cholesterol comprises the transport mechanism used by the body to return excess cholesterol to the liver where it is metabolized.

The two main sources of cholesterol in humans are the intracellular esterification of dietary cholesterol in the gut,[7] and the biosynthesis of cholesterol from acetyl CoA (*via* the mevalonic acid pathway) in the liver.[8] The cholesterol produced by the liver accounts for 60-70% of the total cholesterol content in blood. Regulating the production of this biosynthetic cholesterol is one of the most important areas of current research in the development of pharmaceutical lipid-lowering drugs.

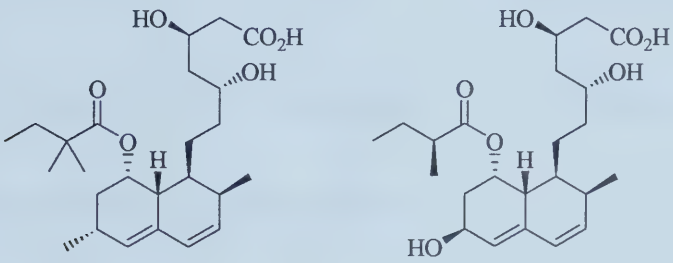
Compactin **2** (ML-236B, mevastatin) was the first natural cholesterol-lowering drug isolated in 1976 from *Penicillium citrinum*[9] and from *Penicillium aurantiogriseum*

(formerly named *Penicillium brevicompactum*).[10] Lovastatin **1** (mevinolin, monacolin K, Mevacor™) was subsequently isolated from *Monascus ruber*[3] and from *Aspergillus terreus*.[2] Both lovastatin **1** and compactin **2** significantly lower the rate of steroid biosynthesis in human cell cultures and in animals.[11] The mode of action for both compounds is by the competitive reversible inhibition of (3*S*)-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase),[12] which is a rate-limiting enzyme that catalyzes the reductive deacylation of HMG-CoA **8** to mevalonic acid **10** during the cholesterol biosynthesis (Scheme 1).



Scheme 1. Reduction of (3*S*)-hydroxy-3-methylglutaryl CoA **8** to mevalonic acid **10** *via* HMG-CoA reductase.

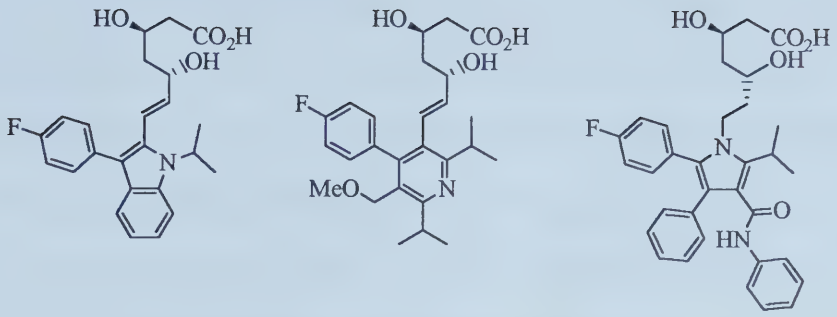
Other cholesterol lowering compounds such as simvastatin **11** (Zocor, Merck)[13] and pravastatin **12** (Sankyo, Bristol-Meyers Squibb)[14] were subsequently developed (Figure 4). These drugs all mimic the intermediate **9** that is formed in the reduction of (3*S*)-hydroxy-3-methylglutaryl CoA **8** to mevalonic acid **10** (Scheme 1).



Simvastatin (**11**) Pravastatin (**12**)

Figure 4. Simvastatin **11** and Pravastatin **12**, two analogues of natural statins.

Other synthetic hypercholestrolemic HMG-CoA reductase inhibitors currently in use are fluvastatin **13** (Lescol, Novartis),[15] cerivastatin **14** (Baycol, Bayer),[16] and atorvastatin **15** (Lipitor, Parke-Davis & Co.)[17] (Figure 5). Although structurally different from lovastatin **1** and compactin **2**, these compounds all possess the opened lactone moiety necessary for inhibition of HMG-CoA reductase. The search for new hypercholesterolemic drugs continues, but the HMG-CoA reductase inhibitors (lovastatin **1**, simvastatin **11** and pravastatin **12**) mentioned above, are still the most effective hypercholestrolemic drugs available with combined sales of $7.53 billion (in 1997).[18]



Fluvastatin (**13**) Cerivastatin (**14**) Atorvastatin (**15**)

Figure 5: Synthetic HMG-CoA reductase inhibitors.

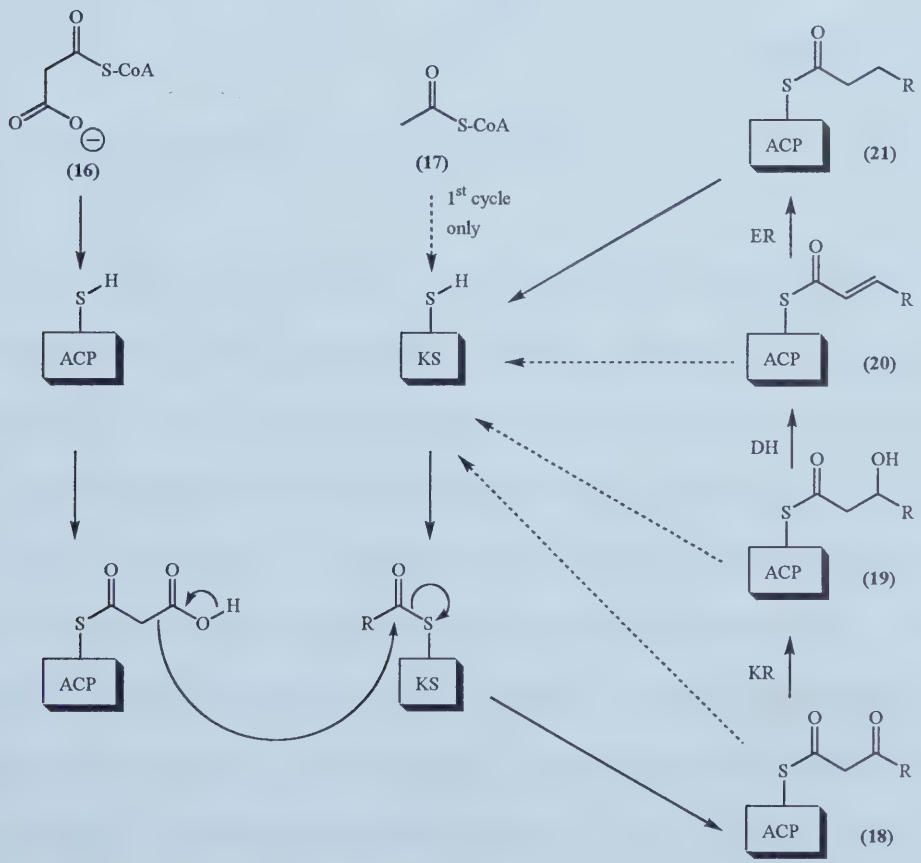
1.2 Polyketide Synthases (PKSs)

1.2.1 General characteristics of PKSs

Despite the structural diversity of polyketides, they are all thought to be assembled in a similar series of steps. PKSs are large multifunctional proteins,[1] consisting of several active sites. The smallest PKSs consist of three active sites, where each active site is responsible for one step in the elongation cycle: acyl carrier protein (ACP), ketosynthase (KS) and acyl transferase (AT).

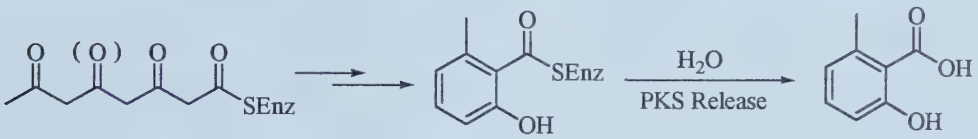
In the first cycle the starter unit (often acetyl CoA **17**) is loaded onto the KS, and the extender unit (commonly malonyl CoA **16**) is loaded onto the ACP (Scheme 2). Decarboxylative condensation of the starter unit onto the acetyl unit affords a *β*-ketoacetyl-*S*-ACP **18**. This unit **18** is sometimes modified by optional domains on the PKS, such as a ketoreductase (KR), dehydratase (DH), enoyl reductase (ER) or methyl

transferase (MeT). The diketide **21** is transferred from the ACP to the active site KS. A second malonate unit **16** is then loaded onto the ACP, and condensed with the diketide **21** to give a triketide, which may again be transformed to the appropriate oxidation level. The cycle may be repeated until the desired chain length is attained. The intermediate oxidation states that are generated on the polyketide acyl chain during each cycle remain unaltered once the polyketide chain is transferred for further elongation.



Scheme 2. General biosynthesis of polyketides.

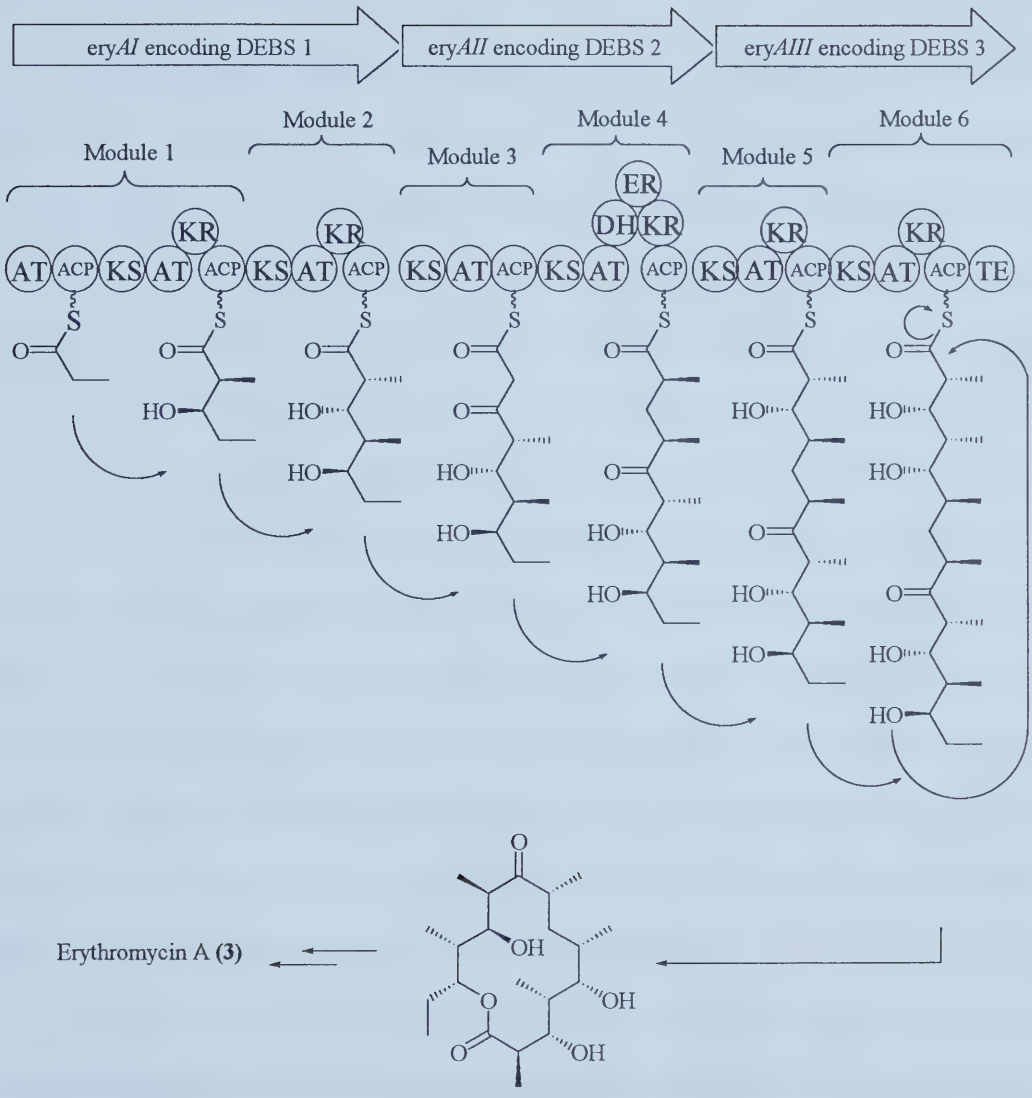
Once the desired chain length is attained, the polyketide is released from the enzyme *via* intermolecular attack by water, or by intramolecular cyclization. An example of the first type of cyclization and release is found in the biosynthesis of 6-methylsalicylic acid (6-MSA) **22**, affording the free acid (Scheme 3) after release from the enzyme.



6-Methylsalicylic acid (**22**)

Scheme 3. Chain termination by water in the biosynthesis of 6-methylsalicylic acid **22**.

The cyclization may sometimes be assisted by the thioesterase (TE) which regenerates the active ACP. Intramolecular cyclization and release by a group, such as a hydroxyl group on the polyketide chain, leads to the formation of a lactone, as is the case for 6-deoxyerythronolide B **23**, the PKS product leading to erythromycin A **3** (Scheme 4). This PKS enzyme system, 6-deoxyerythronolide B synthase (DEBS), which produces 6-deoxyerythronolide B **23**, consists of three large multifunctional proteins, DEBS1, DEBS2, and DEBS3, each organized into two modules. Each module carries the catalytic domains required to generate one extender unit. The product is further modified by post-PKS enzymes, once released from the DEBS enzymes, to yield erythromycin A **3**.



Scheme 4. Biosynthesis of the polyketide component of erythromycin A **3**.[19]

1.2.2 Classification of PKSs

PKSs are often classified according to the arrangement and function of their active sites.[4] Bacterial PKSs consist of either a modular type I or an iterative type II protein, fungal PKSs are iterative type I, and plant PKSs make up the type III class. The modular type I system, exemplified by DEBS, have multiple catalytic sites in a large polypeptide chain with each module being used only once to assemble a single molecule. The iterative type II system responsible for the biosynthesis of tetracenomycin C **4** consists of several separate polypeptides, each containing a single active site, which is used as often as required. The iterative type I PKSs, for example 6-methylsalycylic acid synthase (6-MSAS), contains one active site for each catalytic function, and these active sites are used repeatedly as it is required. The final product can often be deduced in modular enzymes, based on gene sequencing, however this is not the case for iterative systems. The factors determining when and the amount of times each active site of the iterative PKSs is used, remains to be elucidated. Bacterial PKSs of both types (types I and II) have been studied to a greater extent than fungal PKSs.

The type III synthases are very different from all other known PKSs and fatty acid synthases (FASs). Like other synthases they catalyze the linking of two carbon units by repeated decarboxylative condensations. There are however major differences in the process, notably the participation of free CoA esters as substrates. Protein sequence comparison revealed no significant overall resemblance with any PKSs of the other types.[4] The type III family also includes some enzymes that generate tetraketides from starter units like isovaleryl CoA and isobutyryl CoA.

1.3 Lovastatin biosynthesis

1.3.1 Previous research efforts to elucidate the biosynthesis of lovastatin (1).

Vederas and coworkers[20] investigated the biosynthesis of lovastatin **1** by conducting feeding studies *with A. terreus* (using labeled precursors) to determine the origins of the carbon, hydrogen and oxygen atoms.[20,1g] They determined that the basic skeleton of lovastatin is obtained by the combination of a 4-carbon and a 18-carbon polyketide chain, derived from the coupling of acetate units (Figure 6). The methyl groups at the C-6 and C-2' positions originate from *S*-adenosyl-*L*-methionine (SAM).

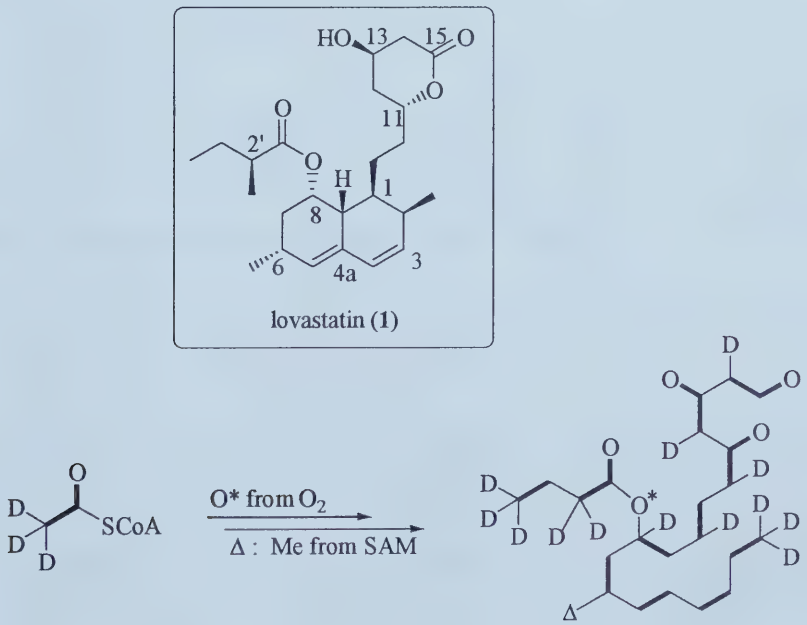
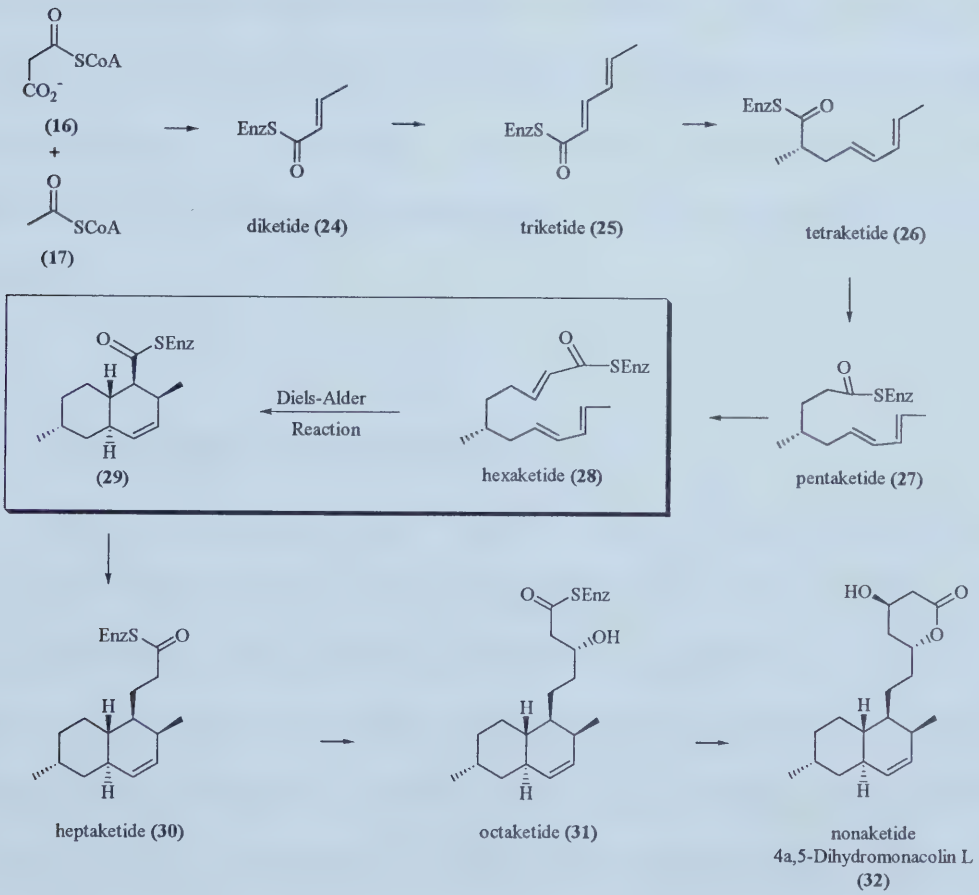


Figure 6. Origins of the carbon, hydrogen and oxygen atoms of lovastatin **1**.

It was also determined that all the oxygen atoms contained in lovastatin **1** are all derived from acetate, with the exception of the oxygen at C-8, which is introduced aerobically.[1g] These labeling studies led to the proposal of a putative biosynthetic pathway of lovastatin **1** (Scheme 5).



Scheme 5. Proposed pathway for the biosynthesis of lovastatin **1**.

The biosynthesis starts with the condensation of acetate **17** and malonate **16**, followed by reduction and dehydration to afford a diketide **24** (Scheme 5). These processes are then repeated several times (with the appropriate transformation at each step by a ketoreductase (KR), dehydratase (DH), enoylreductase (ER), or methyltransferase (MeT)) to produce the hexaketide **28**. The hexaketide **28** is then transformed *via* a putative Diels-Alder reaction to generate the bicyclic decalin adduct **29**, which is then extended to give the octaketide **31**. The octaketide **31** is extended to the nonaketide which is released from the polyketide synthase (PKS) as 4a,5-dihydromonacolin L **32**. Post-PKS transformation of 4a,5-dihydromonacolin L **32** afford lovastatin **1**.

1.3.2 Characterization of the lovastatin genes

Reeves, McAda and coworkers[1a] identified a type I PKS gene responsible for lovastatin **1** biosynthesis from *A. terreus*. This gene was approximately 300 kDa in size and contained the sequence motifs responsible for the expected catalytic functions associated with the biosynthesis of the nonaketide chain of lovastatin **1**. The sequence similarities of these motifs (KS, AT, DH, ER, KR, ACP), and the order in which they are arranged were similar to that observed for other multifunctional polyketide and fatty acid synthases. These observations enabled the identification, cloning and characterization of the LNKS gene, as well as of the other genes involved in lovastatin **1** biosynthesis [21] (Figure 7).

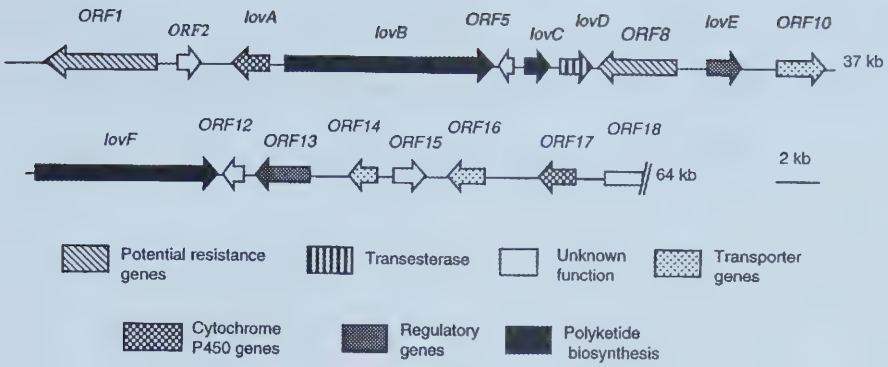
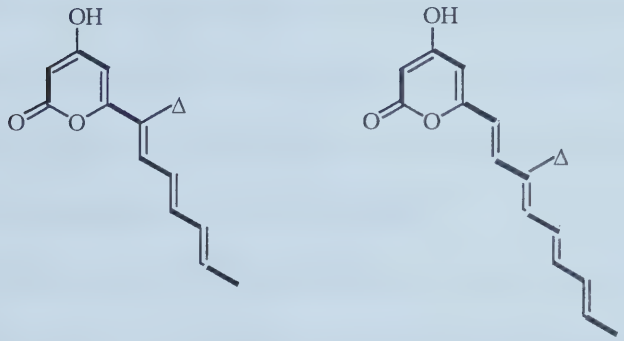


Figure 7. The lovastatin **1** gene cluster.

Two PKS genes (*lovB* and *lovF*) each containing six domains characteristic of PKSs (KS, AT, DH, ER, KR, ACP) along with a MeT domain were found. The *lovF* gene encodes for the lovastatin diketide synthase (LDKS) which is responsible for the biosynthesis of the (2*R*)-2-methylbutyryl side chain. The absence of a thioesterase (TE) domain at the C-terminus of LDKS indicates that the release of the diketide product is done by another enzyme, probably *lovD*, a 46 kDa protein that catalyzes the transfer of the diketide side chain in lovastatin **1**. The *lovE* and *ORF13* genes are thought regulate lovastatin production since the introduction of more copies of *lovE* into the wild-type strain, resulted in a seven to ten fold overproduction of lovastatin **1**.[21] LovA and ORF17, are believed to act as cytochrome P450 proteins employed in post PKS assembly, based on their sequence similarities to known P450 enzymes. Self-resistance to lovastatin **1** is attributed to the *ORF1* and *ORF8* genes.

Studies with LovB (after cloning and overexpression into the heterologous host *Aspergillus nidulans*) revealed the formation of two new polyketides, namely 4-hydroxy-6-[(1*E*,3*E*,5*E*)-1-methylhepta-1,3,5-trien-1-yl]-2-pyrone **33**, and 4-hydroxy-16-[(1*E*,3*E*,5*E*,7*E*)-3-methylnona-1,3,5,7-tetraen-1-yl]-2-pyrone **34**.[19,21]



(33) **(34)**

Figure 8. Pyrones **33** and **34** produced by the LovB protein, with methyl (Δ) derived from SAM.

These pyrones originate from acetate, and are indicative of a malfunctioning enoyl reductase (ER) domain within the PKS. The presence of the SAM derived methyl group in both these compounds indicates that the MeT domain is still active, and acts regioselectively. The enzyme is however unable to catalyze further reactions correctly once the ER reaction fails to occur at the tetraketide stage.

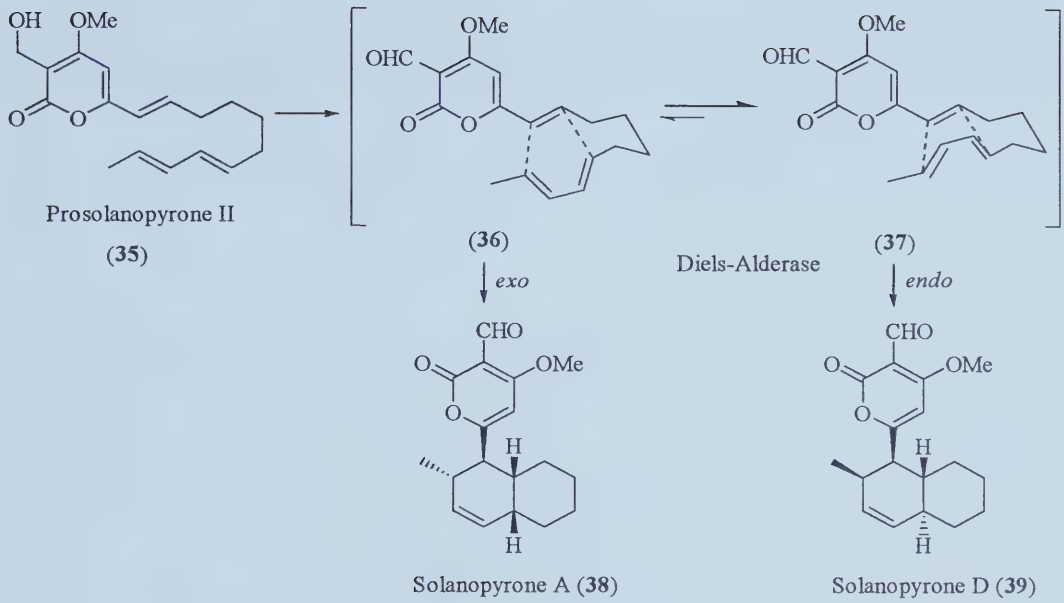
A second ER containing enzyme, probably lovC, is therefore required to produce dihydromonacolin L. This was confirmed with the isolation of dihydromonacolin L after co-expression and testing of lovB and lovC in *A. nidulans*.[21]

1.3.3 Diels-Alder activity of lovB

It has been reported that cell-free extracts of *Alternaria solani* produce a conjugated triene **35**, which after oxidation of the alcohol functionality affords a triene aldehyde **36** which undergoes an intramolecular Diels-Alder cyclization, to afford solanopyrone A **38** and solanopyrone D **39** (Scheme 6).[22]

A closer examination of the stereochemistry of 4a,5-dihydromonacolin L **32** suggests that a Diels-Alder reaction might be responsible for the formation of the decalin ring system from the hexaketide **28** (Scheme 5). Similar transformations have been proposed for the biosynthesis of other secondary metabolites[23] and have been demonstrated for catalytic antibodies generated from synthetic haptens, [24] as well as for synthetic RNA fragments which bind metals.[25] A Diels Alder reaction has also been reported to occur with a cell-free extract of *Alternaria solani* during the biosynthesis of solanopyrone A[26] (Scheme 6).

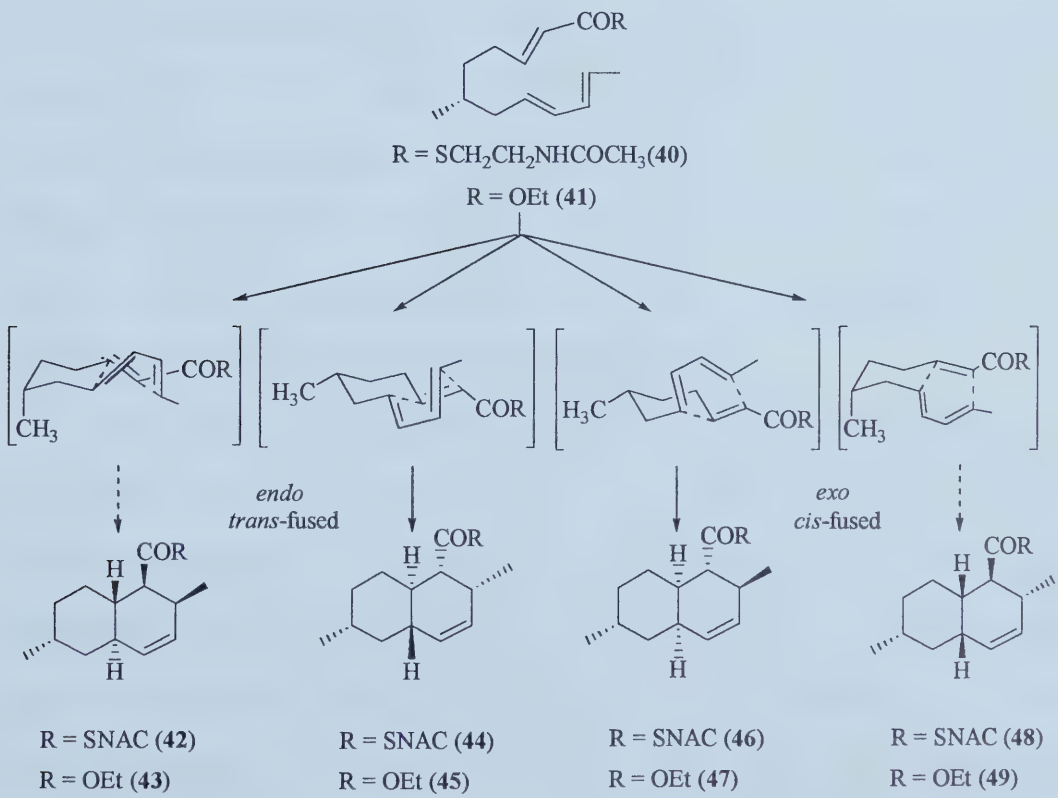
The initial cyclization studies of the putative Diels-Alder reaction in the lovastatin **1** biosynthesis were conducted by Witter and Vederas with the *N*-acetylcysteamine (NAC) ester derivative of the triene hexaketide **40** (Scheme 7).[1h]



Scheme 6. Diels-Alder cyclization during the biosynthesis of solanopyrone A **38** and solanopyrone D **39**.

The cyclization of this hexaketide can be envisioned to occur *via* four possible transition states (2 *exo* and 2 *endo*). Thermal cyclization of the triene NAC ester **40** yielded a mixture of compounds **44** and **46**, while thermal cyclization of the corresponding ethyl ester **41** give products **45** and **47**. The formation of these products result from cyclization *via* the transition states where the C-6 methyl group occupies a sterically favored pseudo equatorial orientation. Formation of the decalin system **42**, which has the same stereochemistry as 4a,5-dihydromonacolin L **32**, requires an *endo* transition state, with the C-6 methyl group in a pseudo axial orientation. The formation of **42** was never observed in non-enzymatic, thermal or Lewis acid catalyzed reactions. A

labeled version of the triene hexaketide NAC ester was incubated with growing cultures of *A. terreus* under various experimental conditions to test whether this molecule could be incorporated into lovastatin **1**. No incorporation of labeled material was detected in the resulting lovastatin **1**, probably due to the rapid degradation of the starting material by *A. terreus*.



Scheme 7. Cyclization the *N*-acetylcysteamine (NAC) ester derivative **40** of the triene hexaketide.

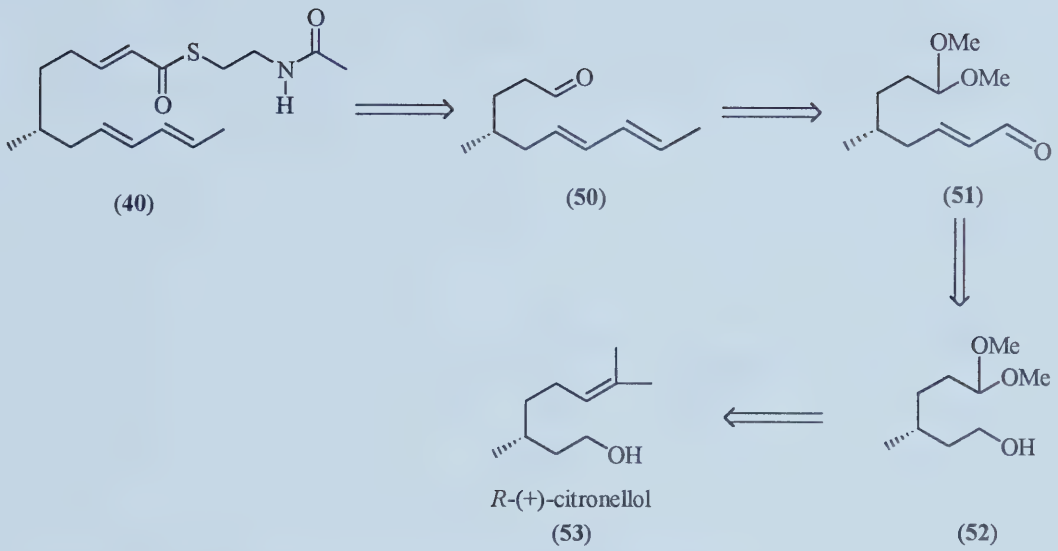
2 Results and discussion

2.1 Synthesis of possible substrates for putative enzymatic Diels Alder studies.

2.1.1 Synthesis of methylated hexaketide derivatives.

It was envisioned that an investigation into the putative Diels-Alder reaction catalyzed by the lovB protein could be undertaken with the *N*-acetylcysteamine analogue of the hexaketide **40** (Scheme 8). If this approach proved to be successful further analogues would be synthesized in order to determine the selectivity of the enzyme for different substrates. Testing of the compactin hexaketide analogue, which is not methylated at the C-6 position, would then also be attempted.

The synthesis was conducted largely according to the method of Witter and coworkers.[1h] The synthesis started with *R*-(+)-citronellol **53**, which sets the stereochemistry at the C-6 position. This is a commercially available starting material that is functionalyzed at both ends, leading to a two-directional approach to the final product. This approach is outlined in the retrosynthetic analysis (Scheme 8), and involved successive formation of aldehydes, which can be transformed by Wittig chemistry to yield the desired alkene **51**. Deprotection of the acetal, followed by elongation of the chain using Wittig chemistry would yield the desired product **40**.



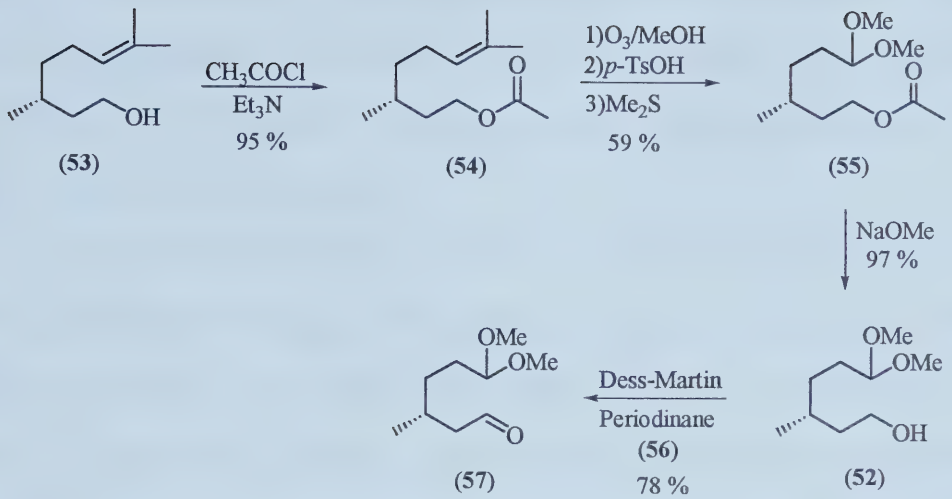
(40) **(50)** **(51)**

R-(+)-citronellol
(53)

(52)

Scheme 8. Retrosynthetic analysis of *N*-acetylcysteamine (NAC) hexaketide **40** synthesis.

Initially the alkene in *R*-(+)-citronellol **53** needs to be converted to an acetal. Therefore, the alcohol was first protected with acetyl chloride and triethylamine as the base, to give the ester **54** (Scheme 9).



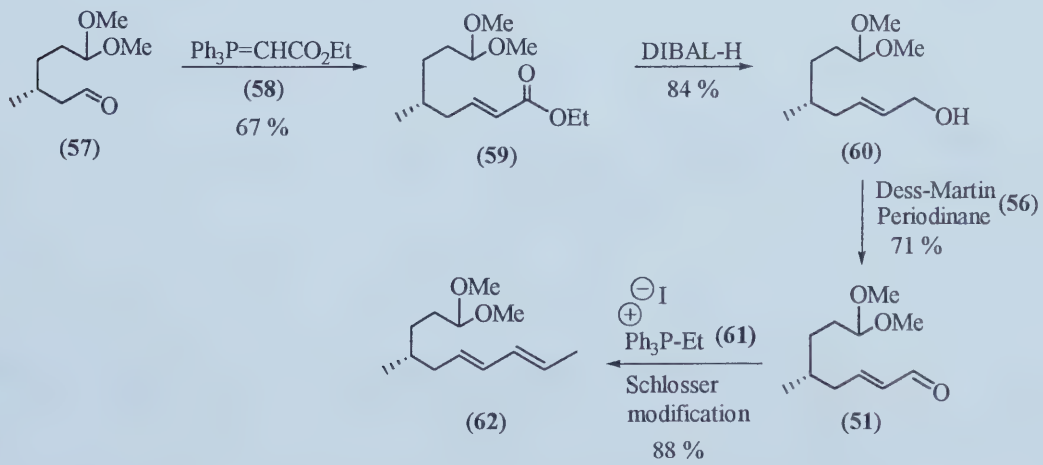
Scheme 9. Synthesis of aldehyde **57** from *R*-(+)-citronellol **53**.

The original synthesis by Witter[1h] involves ozonolysis of the double bond to obtain an aldehyde, which is then protected as the acetal. Removal of water is achieved by a using a Dean Stark apparatus. However, an alternative one-pot method can be employed (Scheme 9). This method involves ozonolysis of **54** in methanol, followed by overnight treatment with *p*-TsOH, and then reduction of the resulting peroxide with dimethyl sulfide to yield the desired acetal **55** in modest yield (59 %). This method is more convenient since it requires one less step, and it results in a similar overall yield of acetal **55** compared to that obtained by Witter.

Once acetal **55** is obtained, hydrolysis of the acetate is achieved by treatment with sodium methoxide in methanol, to give alcohol **52** (97 %). Oxidation of alcohol **52** with Dess-Martin periodinane **56** affords aldehyde **57** in good yield (78 %). Dess-Martin reagent **56** is preferred for all oxidation reactions of alcohols due to the short reaction

time of 30 min, and the mild reaction conditions required (room temperature). The Dess-Martin periodinane **56** was initially prepared by the original method described in the literature,[27] but subsequently an alternative approach was used which is safer and which avoids bromine gas formation.[28]

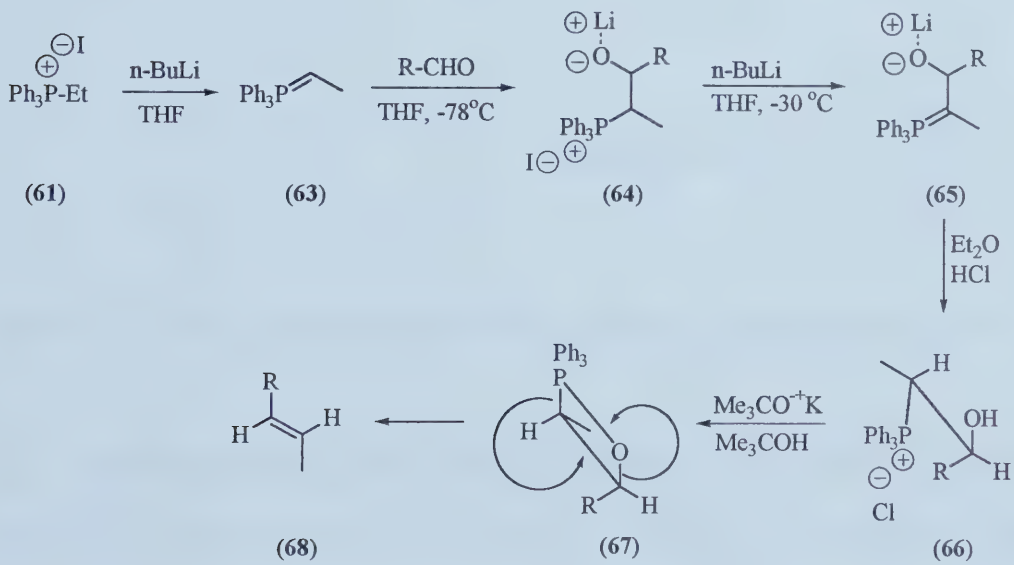
The first alkene **59** is obtained using Wittig chemistry (Scheme 10). The aldehyde **57** is treated with the Wittig reagent **58**, to afford a mixture of *E*- and *Z*-alkenes, with the *E*-alkene **59** being the major product. After isolation and purification of the *E*-alkene **59**, the ester functionality is reduced to the corresponding alcohol **60** using DIBAL-H. Oxidation of this alcohol **60** with Dess-Martin periodinane **56** affords the aldehyde **51** in good yield. The second double bond is then introduced using the Schlosser modification of the Wittig reaction (Scheme 10), to afford the diene **62**.



Scheme 10. Synthesis of diene **62** from aldehyde **57**.

The use of the Schlosser modification is necessary since ylids that contain triarylphosphines, but have no stabilizing groups, often produce predominantly *Z*-alkenes.

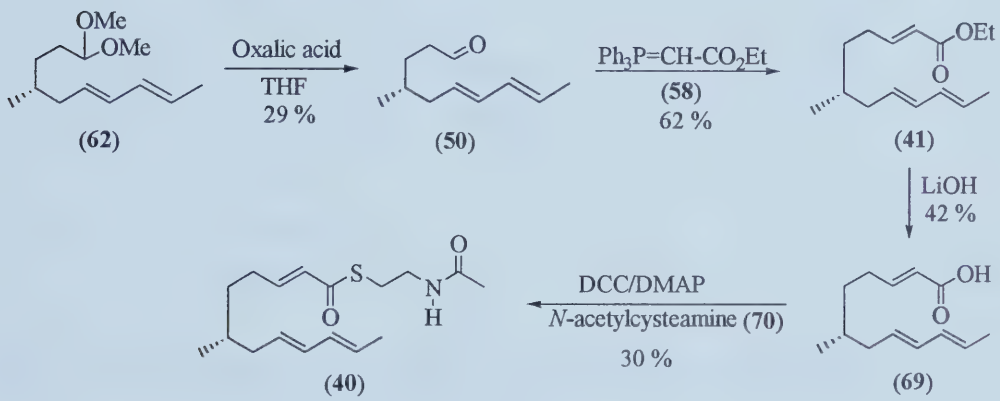
In the reaction used to form diene **62** (Scheme 10) a standard Wittig reaction would predominantly form the *Z*-alkene, whereas the *E*-alkene is required. The Schlosser modification (Scheme 11) involves the use of a strong base (n-BuLi) to deprotonate the betaine-lithium iodide adduct **64**, and the subsequent addition of a proton source (*e.g.* ethereal HCl) to generate the more stable *threo*-betaine-like adduct **66**. Addition of potassium t-butoxide then liberates the *E*-olefin **68** preferentially.



Scheme 11. The Schlosser modification of the Wittig reaction.

As indicated above, the use of Schlosser conditions with the aldehyde **51** (Scheme 10) and phosphonium salt **61** produces the 2*E*,4*E*-diene **62** in good yield, with only trace amounts of the 2*Z*,4*E*-isomer.

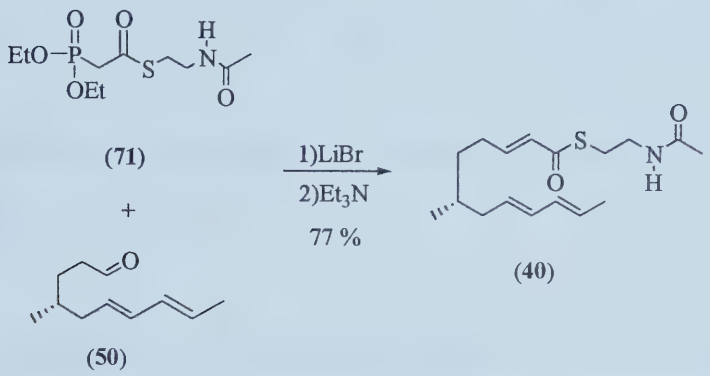
The next steps in the synthesis involves the conversion of the acetal **62** (Scheme 12) to the aldehyde **50** by treatment with a saturated solution of oxalic acid in THF overnight at room temperature. The aldehyde **50** is very volatile, and this creates difficulties during purification, resulting in low yields (29 %).



Scheme 12. Synthesis of the desired target compound **40** *via* the acid intermediate **69**.

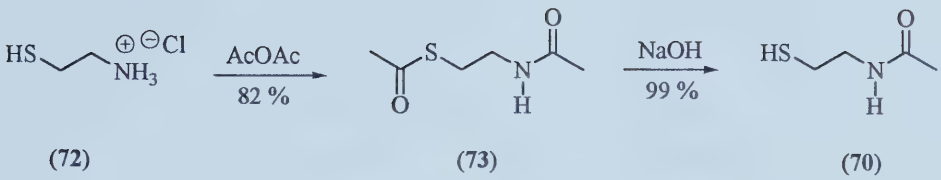
The original method used by Witter[1h] to construct the desired NAC-ester **40**, involves reaction of the aldehyde **50** with the Wittig reagent **58** to obtain the ethyl ester **41**. The ethyl ester **41** is then converted to the corresponding acid **69** by treatment with aqueous LiOH in THF, followed by acidification to pH 7 with HCl (Scheme 12). A problem with this approach is the instability of acid **69** during the acidic work up and purification. Decomposition and Diels-Alder cyclization of the acid **69** results in poor yields (30 %). In the Witter approach the acid **69** is then coupled to *N*-acetylcysteamine **70** using dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP), to afford the desired *N*-acetylcysteamine ester (NAC) **40** in very low overall yield (Scheme

12). A more elegant approach to obtain the NAC-ester **40** (Scheme 13) involves reaction of aldehyde **50** with Wittig reagent **71**. This results in a much higher yield (77 %) in fewer steps, and eliminates the unstable acid **69** from the synthesis.



Scheme 13. Alternative synthesis of NAC-ester **40** using Wittig reagent **71**.

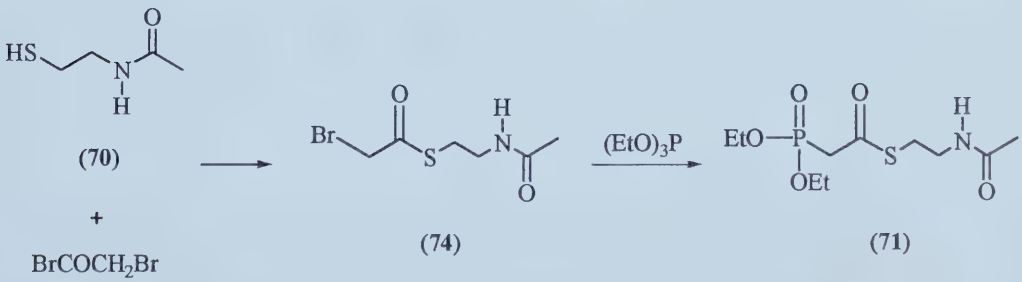
N-Acetylcysteamine **70** is prepared using a literature procedure[44] (Scheme 14) where 2-mercaptoethylamine hydrochloride **72** reacts with acetic anhydride to give the diacetate **73**. Selective deprotection of the acetate group gives *N*-acetylcysteamine **70**.[43]



(72) (73) (70)

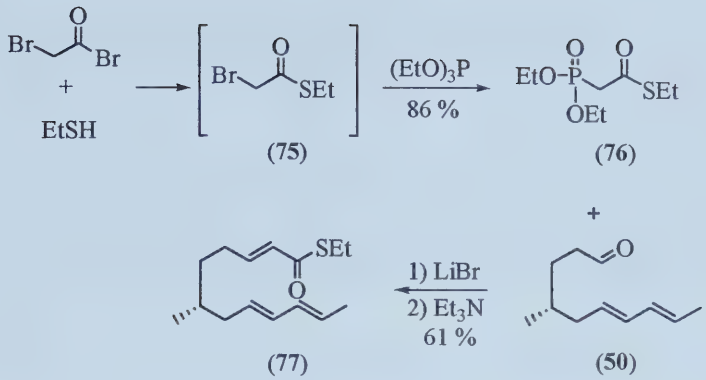
Scheme 14. Synthesis of *N*-acetylcysteamine **70** from 2-mercaptoethylamine hydrochloride **72**.

The Wittig reagent **71** is obtained by reaction of bromoacetyl bromide with *N*-acetylcysteamine **70** to give the bromide **74**. The bromide **74** is then heated under reflux in toluene with triethyl phosphite, resulting in the formation of the desired Wittig reagent **71** (Scheme 15).



Scheme 15. Preparation of the Wittig reagent **71** from *N*-acetylcysteamine **70**.

Using the same methodology as above (Scheme 15), the thioethyl Wittig reagent **76** is prepared and used to synthesize the thioester derivative 77 (Scheme 17).



Scheme 17. Synthesis of thioester **77** from aldehyde **50** using Wittig reagent **76**.

The synthesis of the target compound **40**, as well as two other analogues, namely the ethyl ester **41** and thioester **77** is thus achieved (Figure 9). These three compounds were used in subsequent biological and model studies.

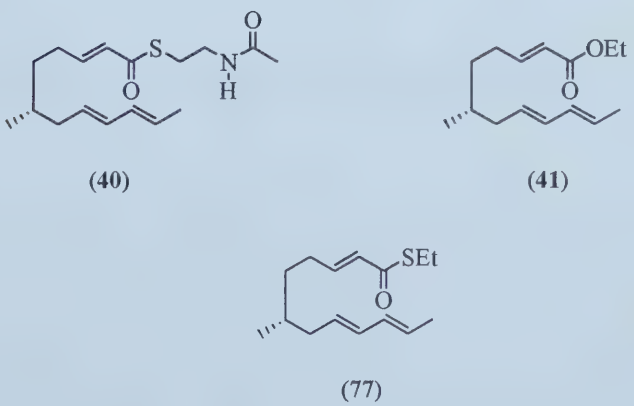
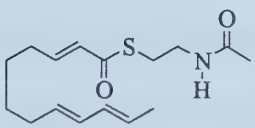


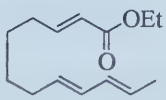
Figure 9. Hexaketide analogues **40**, **41** and **77** for biological testing.

2.1.2 Synthesis of compactin analogues for enzyme studies.

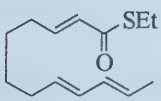
The next stage of these studies involved the preparation of three analogues with similar structures to the methylated hexaketide derivatives (Figure 9), but without the methyl groups.



(78)



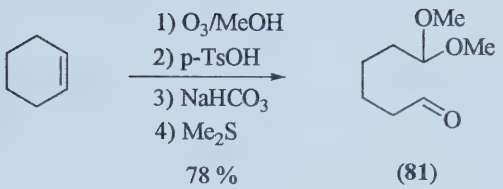
(79)



(80)

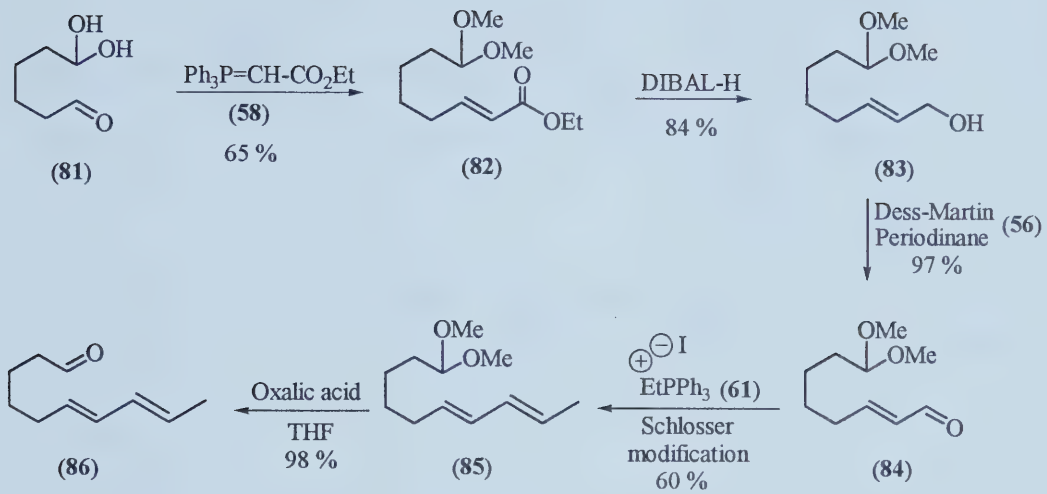
Figure 10. Compactin related hexaketide analogue compounds **78**, **79** and **80**.

The synthesis of the target compounds **78**, **79** and **80** is accomplished following the same general methodology as discussed above for the lovastatin series. Synthesis of the bi-functional compound **81** can be achieved in a one-pot reaction starting from cyclohexene (Scheme 18). Ozonolysis of cyclohexene, and treatment with *p*-TsOH give a mixture that is treated with sodium bicarbonate followed dimethyl sulfide, to reduce the peroxide that forms. This affords the desired product **81** in 78 % yield.



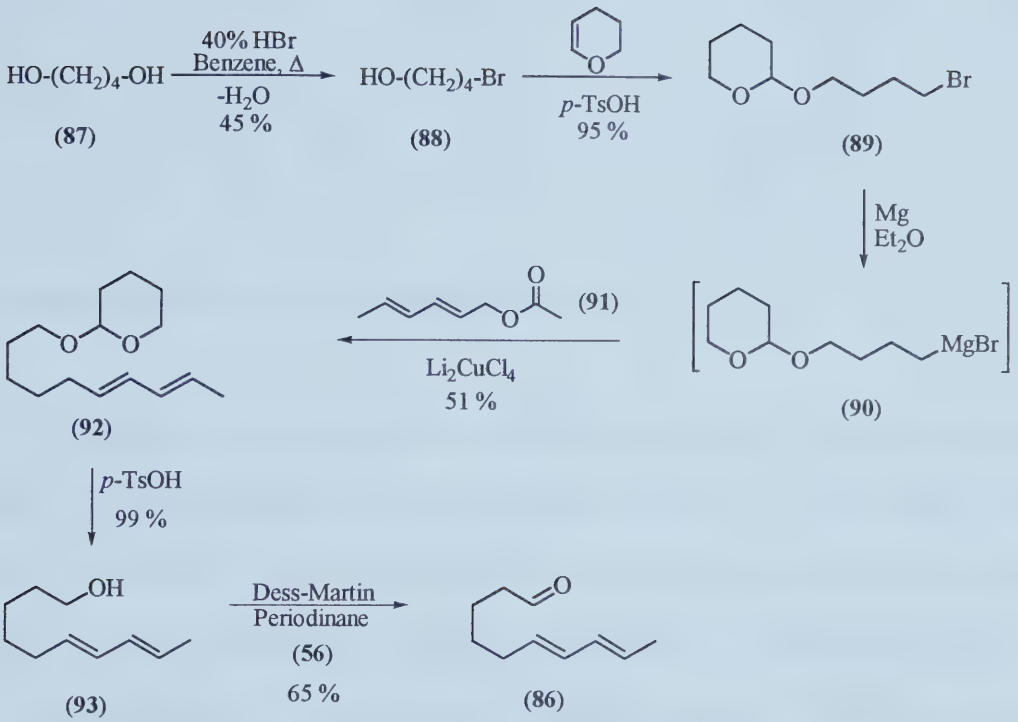
Scheme 18. Synthesis of the bifunctional compound **81** from cyclohexene.

The synthesis of aldehyde **86** (Scheme 19) is then possible in a similar fashion to that discussed for the methylated hexaketide analogues. Aldehyde **81** is heated under reflux overnight with the Wittig reagent **58** to yield predominantly the *E*-ethyl ester **82**. The ethyl ester **82** is treated with DIBAL-H at –78 °C in dichloromethane to give the alcohol **83**. Oxidation of this alcohol **83** with Dess-Martin periodinane **56** affords aldehyde **84** in quantitative yield. Treatment of aldehyde **84** with the Wittig reagent **61**, using the Schlosser modification of the Wittig reaction, generates the diene **85**. Removal of the acetal with oxalic acid in THF and water gives the desired aldehyde **86**.



Scheme 19. Synthesis of aldehyde **86** from aldehyde **81**.

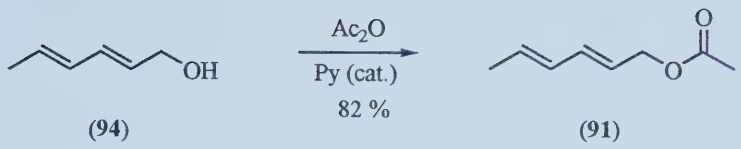
This synthetic route is based on an approach to generate isotopically labeled compounds, and is inefficient and time consuming. Thus, an alternative route to the aldehyde **86** was developed (Scheme 20). Aldehyde **86** could be prepared starting from 1,4-butanediol **87**, which is selectively monobrominated by heating under reflux in benzene with 40% aqueous hydrobromic acid. The alcohol functionality of the resulting bromo alcohol **88** is then protected as the tetrahydropyran (THP) ether **89** using 3,4-dihydropyran and a catalytic amount of *p*-TsOH.



Scheme 20. Alternative synthesis of aldehyde **86** from 1,4-butanediol **87**.

This bromide **89** is converted *in situ* to a Grignard reagent **90**, which reacts with the ester of sorbyl alcohol **91** to yield the THP protected diene **92**.[50] Removal of the THP group can be accomplished by stirring diene **92** in THF with a catalytic amount of *p*-TsOH to obtain alcohol **93**. Oxidation of alcohol **93** with Dess-Martin periodinane **56** affords the aldehyde **86** in good yield (65 %).

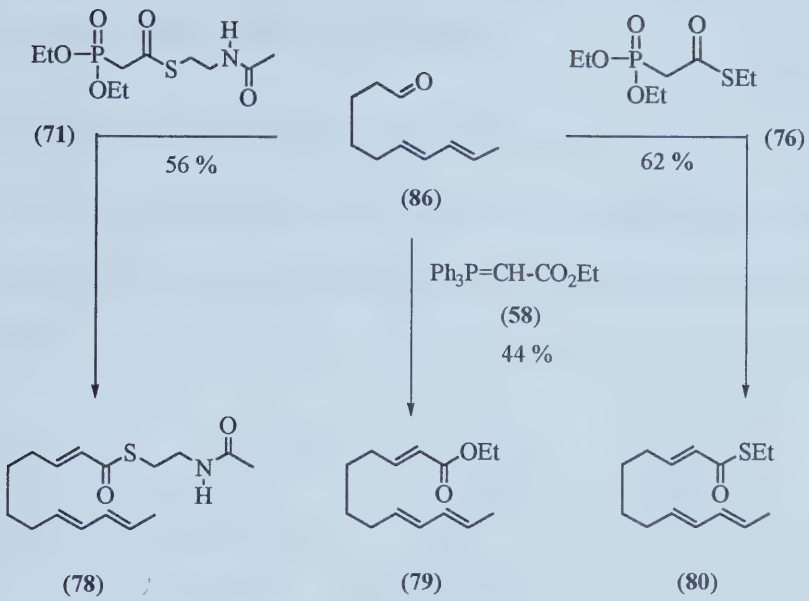
Ester **91** is commercially available, or can be prepared by the reaction of sorbyl alcohol **94** and acetic anhydride, in the presence of a catalytic amount of pyridine (Scheme 21).[51]



(94) (91)

Scheme 21. Synthesis of ester **91** from sorbyl alcohol **94**.

The final target compounds **78**, **79** and **80** are accessible by the same strategy used to synthesize the methylated hexaketide triene analogues. Reaction of aldehyde **86** with the respective Wittig reagents (Scheme 22) affords the NAC ester **78**, thioester **79** and ethyl ester **80** which are used in further biological testing and model studies. With all the compounds needed for the evaluation of the putative enzymatic Diels-Alder reaction for both the lovastatin and compactin in hand, suitable analytical techniques and methods to perform testing of the enzymatic Diels-Alder hypothesis had to be developed. Reference compounds were also needed for unambiguous identification of expected enzymatic products.

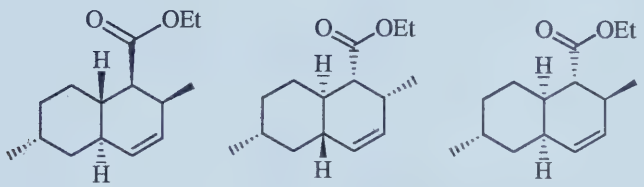


Scheme 22. Synthesis of the three compactin analogues **78**, **79** and **80** from aldehyde **86**.

2.2 Enzymatic studies with the LovB enzyme.

2.2.1 Analysis and testing of the lovastatin analogues.

Reference compounds **43**, **45** and **47** (which are needed to analyze the products of the lovastatin series of analogues) were previously prepared in our group by Dr. D.J. Witter.[1h, 29]



(43) (45) (47)

Figure 11. Reference compounds **43**, **45** and **47** previously prepared in our group.

2.2.2 Testing of (6*R*)-*E,E,E*,-6-methyldodeca-2,8,10-trienoic acid *N*-acetylcysteamine thioester (40) with lovB enzyme.

Incubation of ester **40** with LovB enzyme was done in collaboration with Dr. A. Sutherland. Extraction of the organic components of the mixture and subsequent treatment of the extract with sodium ethoxide to convert the thioesters to ethyl esters afforded a mixture of compounds. Preparative TLC of the mixture yielded a decalin (0.1 mg, 2% over 2 steps) which showed spectroscopic properties (^{1}H NMR, MS) of the reference ethyl ester **43** (Figure 10). A mixture of the ethyl esters **45** and **47** (3.2 mg, 60% over 2 steps) were also isolated. It is noteworthy that the stereochemistry of the isolated

compound **43** is analogous to that of the hexaketide **29** proposed as an intermediate in lovastatin **1** biosynthesis, whereas compounds **45** and **47** correspond to the stereochemistry of the thermal Diels Alder products.

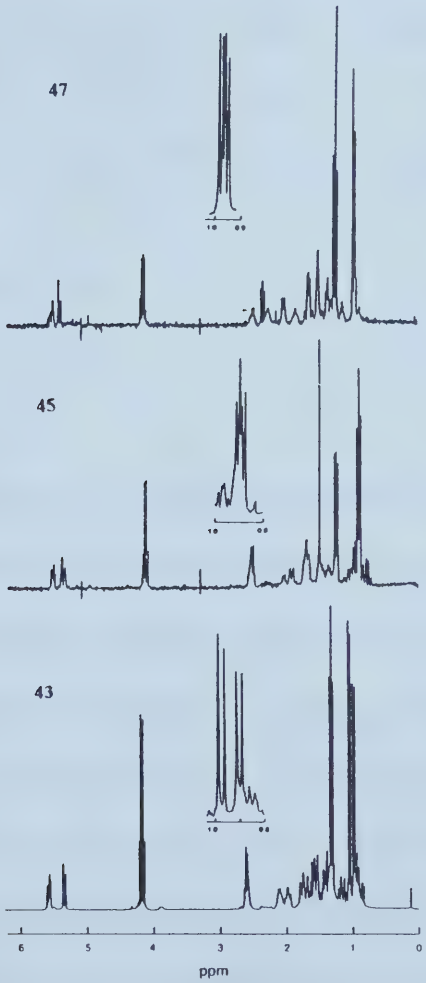
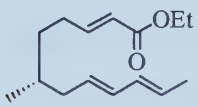
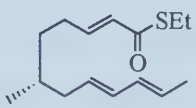


Figure 12. Comparison of the ^{1}H NMR spectra for the three products **43**, **45** and **47** isolated from the enzymatic test mixture.

This spectroscopic evidence strongly suggests that LNKS catalyzes an intramolecular Diels-Alder reaction as part of the biosynthetic pathway to the formation of lovastatin **1**. The same test was repeated with denatured LovB enzyme, and the results showed a mixture of the ethyl esters **45** and **47** (1.9 mg, 62%), with no detectable amount of ester **43**. This indicates that the catalytic activity originates from the active LovB enzyme, and not from a chiral protein surface or from any other components of the buffer solution.



(41)



(77)

2.2.3 Testing of ethyl (6*R*)-(*E*,*E*,*E*)-6-methyldodeca-2,8,10-trienoate (41) and (6*R*)-(*E*,*E*,*E*)-methyldodeca-2,8,10-trienethioic acid *S* ethyl ester (77) with lovB.

Testing and analysis of the ethyl ester **41** and the thioester 77 was conducted in the same manner as for the NAC-ester **40**. Treatment of the respective mixtures obtained after reaction with sodium ethoxide yields a mixture of ethyl esters **45** and **47**, but no detectable presence of ester **43** could be observed. These two compounds (**41** and 77) are therefore not significant as substrates for the LovB enzyme, as control experiments show that **45** and **47** are formed without the presence of the enzyme.

The enzymatic analysis of the compactin series of analogues could not be as easily accomplished as for the lovastatin series, due to the lack of the stereogenic center afforded by the methyl group in the C-6 position. Reference compounds had to be synthesized and a new method of analysis had to be developed. A convenient analytical

method is gas chromatography using a chiral column and a mass spectrometer as the detector (GC-MS).

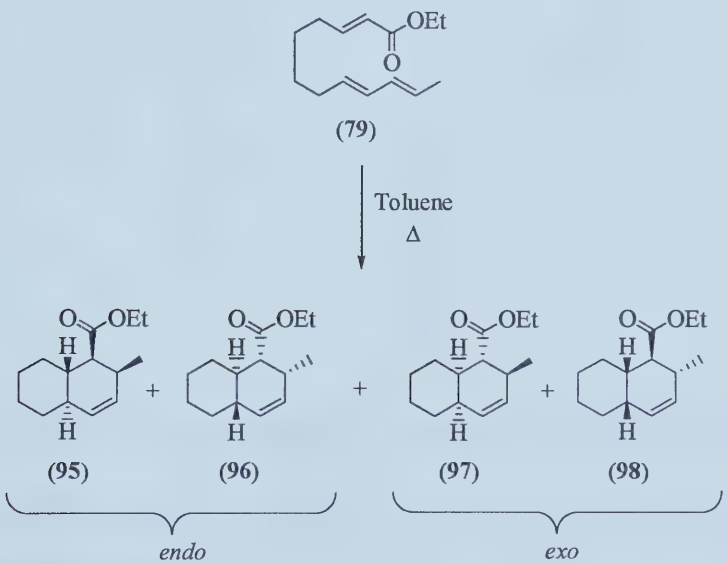
2.2.4 GC-MS method development

2.2.5 Choice of GC column for use in analysis.

The column used in the GC-MS analysis was the β-DEX 120 supplied by Supelco Inc. Supelco supplies three basic columns containing permethylated cyclodextrin embedded in an intermediate polarity stationary phase. Cyclodextrins are cyclic, chiral macromolecules containing six or more D(+)-glucose residues bonded together through α-(1-4) glycosidic linkages, and are classified according to the number of D(+)-glucosides that they contain, *i.e.* α-cyclodextrin (six glucose residues, α-DEX columns), β-cyclodextrin (seven glucose residues, β-DEX columns), and γ-cyclodextrin (eight glucose residues, γ-DEX columns). Cyclodextrin forms a cone shaped structure with the hydroxyl groups outside of the cavity, and the glycosidic oxygens on the inside. The mechanism of separation is not clearly understood, but hydrogen bonding and the size of the cyclodextrin used is important. The β-DEX columns are versatile and are widely used to separate esters, ethers, halohydrocarbons, hydrocarbons, positional isomers, silanes, terpenes and terpineols. A β-DEX 120 column was therefore to analyze ethyl esters formed by possible enzyme-catalyzed (lovB) Diels Alder reaction.

2.2.6 Conditions used for analysis and preparation of standards.

The triene ethyl ester **79** was heated under reflux in toluene for five days to yield a mixture of the four decalins **95**, **96**, **97** and **98** (Scheme 23).



Scheme 23. Synthesis of a mixture of the four decalins **95**, **96**, **97** and **98** *via* thermal cyclization of ester **79**.

This mixture was subjected to GC-MS analysis using the *β*-DEX 120 column. All four isomers could be separated as shown in Figure 13.

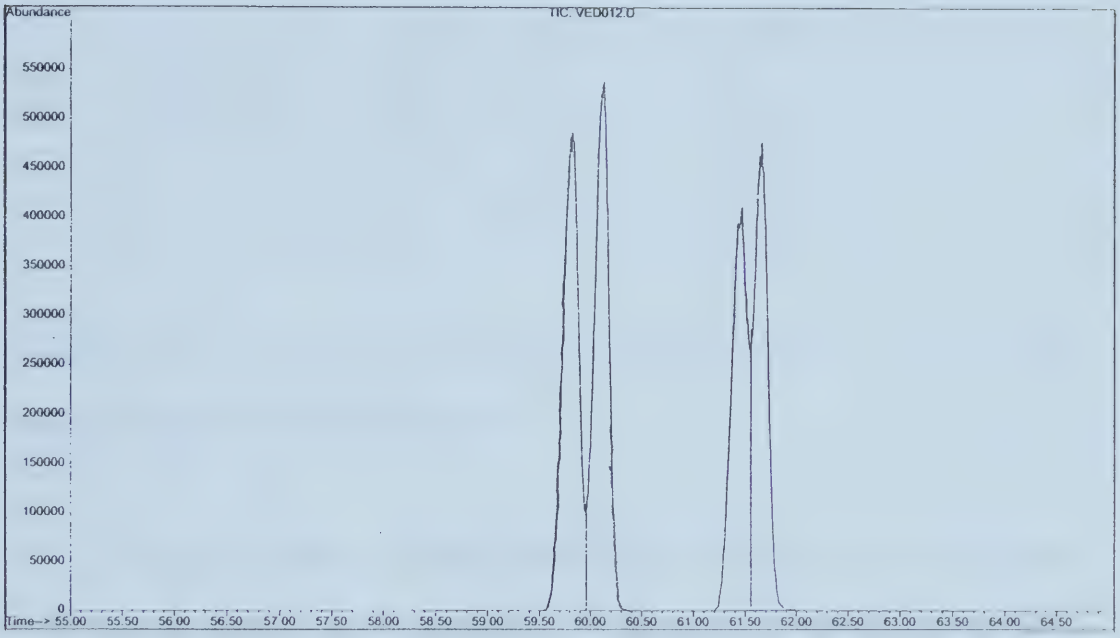
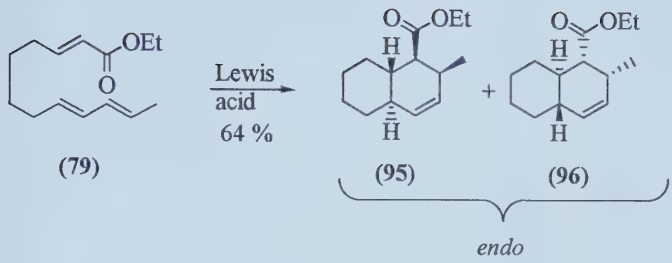


Figure 13. GC analysis of a mixture of the four decalins **95**, **96**, **97** and **98**. For conditions see experimental.

Since Lewis acid catalyzed cyclization reactions afford the *endo* adduct as the major product, the identity of the two *endo* decalins in this mixture (**95** and **96**) could be established by the synthesis of the predominantly *endo* cyclized ethyl esters *via* reaction of the triene ethyl ester **79** with ethyl aluminum chloride (Scheme 24).

GC-MS analysis of this mixture of predominantly endo adducts (Figure 13) gives good separation of two compounds in a 1 : 1 ratio, with retention times of 61.2 min (**95** or **96**) and 61.4 min (**95** or **96**).



Scheme 24. The predominantly endo products **95** and **96** afforded by the Lewis acid catalyzed cyclization of the ethyl ester **79**.

Distinguishing between the *endo* cyclized adducts **95** and **96** can be accomplished by independent synthesis of enantiomer **95** or **96** (Scheme 25). This synthesis employs oxazolidinone chemistry. The synthesis begins with the preparation of a suitable Wittig reagent **101.** The Wittig reagent **101** is prepared by reaction of *R*-(+)-oxazolidinone **99** with bromoacetylbromide to give bromide **100**. The bromide **100** reacts with triphenylphosphine in THF to give the intermediate **101**, which is immediately reacted *in situ* with aldehyde **86** to afford the desired compound **102** in good yield (Scheme 25). Lewis acid cyclization of compound **102**, followed by treatment with sodium ethoxide in ethanol yields predominantly the decalin **96**, as well as minor amounts of the decalins **95**, **97** and **98** (Scheme 25).

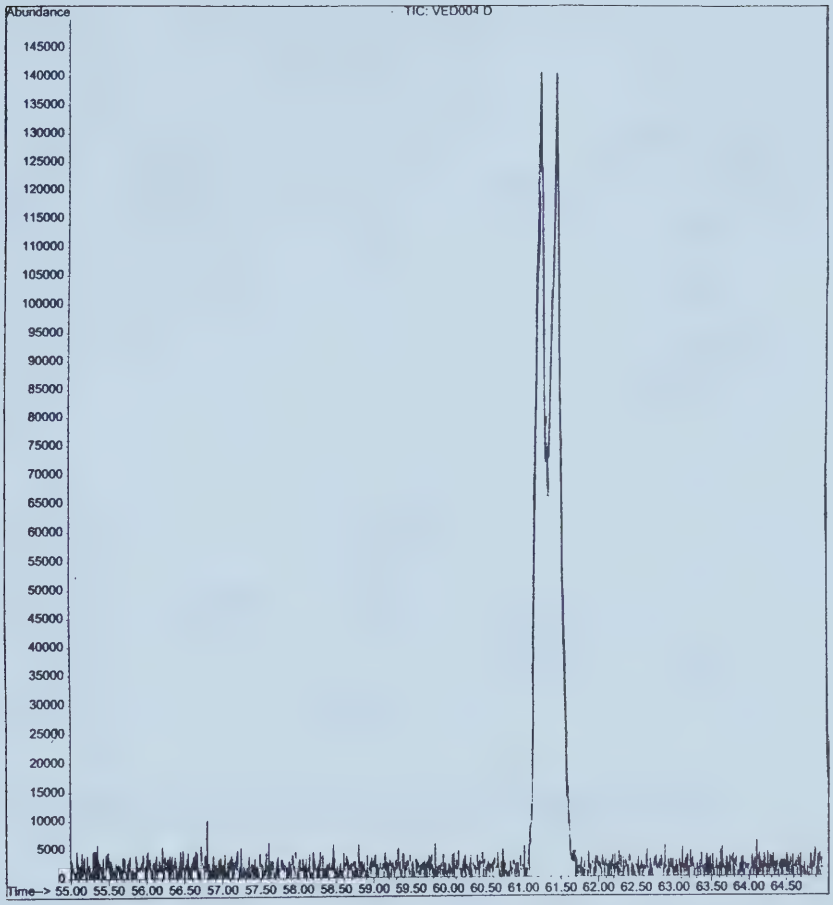
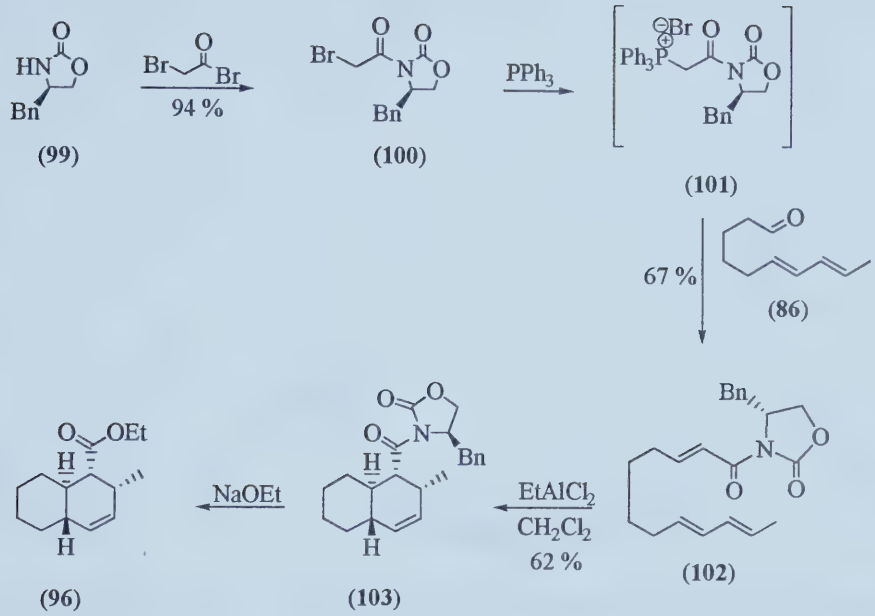


Figure 14. GC-MS analysis of the Lewis acid catalyzed mixture of predominantly endo cyclized products **95** and **96**.



Scheme 25. Synthesis of enantiomer **96** using oxazolidinone chemistry.

GC-MS analysis of the mixture obtained after the Lewis acid cyclization (Figure 14) shows four compounds eluting in a 1 : 1 : 3.5 : 1 ratio with retention times of 60.4 min (**97** or **98**), 60.6 min (**97** or **98**), 61.6 min (**96**), 61.9 (**95**). The fourth compound that elutes from the column is therefore the expected enzymatic product **95**, based on well-established literature precedent for the stereochemistry of such oxazolidinone assisted cyclizations.[30]

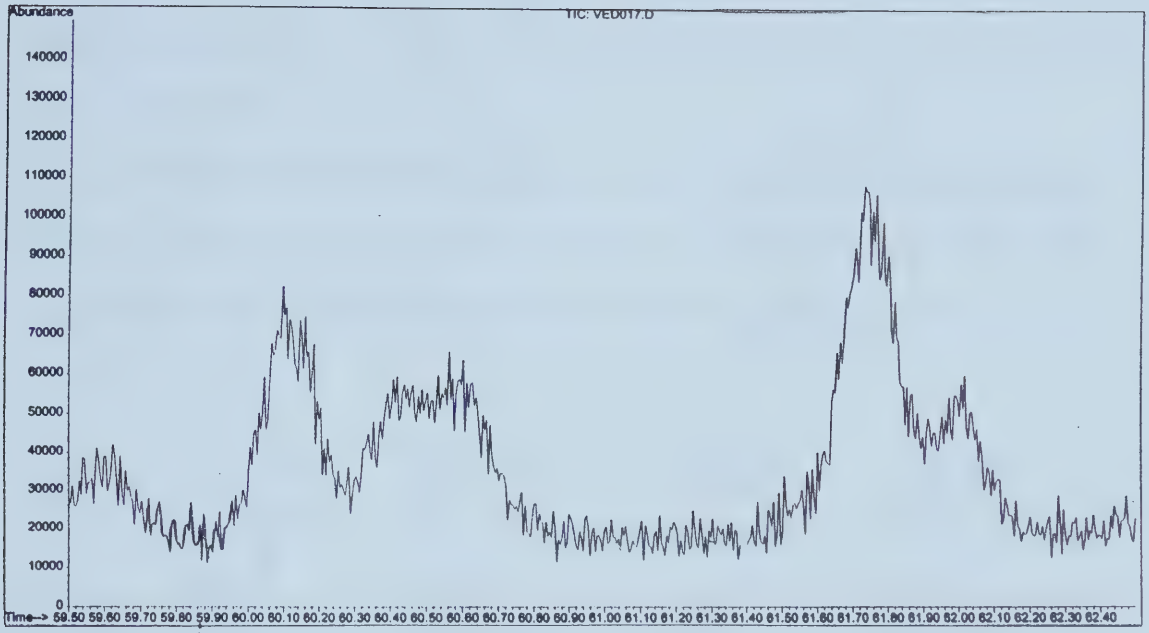
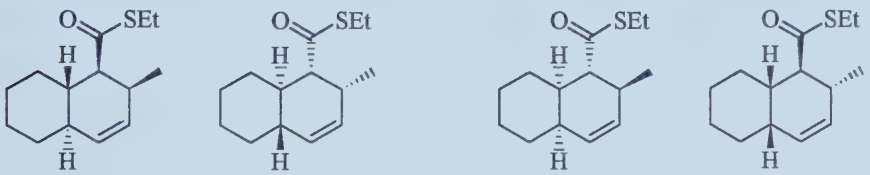


Figure 15. GC-MS analysis of the mixture containing **96** as the major product.

A good method for the analysis of the enzymatic Diels-Alder reaction is thus available. It is however possible that small amounts of uncyclized ethyl ester **79** in the enzymatic test solution (after treatment with sodium ethoxide) might influence the GC-MS results. The chiral environment in the column, combined with the high temperatures used could facilitate the formation of one of the four decalins (**95**, **96**, **97** and **98**) preferentially by Diels-Alder cyclization. A small amount of the uncyclized triene ethyl ester **79** was therefore subjected to GC-MS analysis. A small amount of cyclization is observed (ca 1%), but all four decalins **95**, **96**, **97** and **98** are formed in a 1:1:1:1 ratio.

Any uncyclized material present in the enzymatic test mixtures will therefore not significantly affect results obtained. Analysis of the compactin series of compounds could now be attempted.

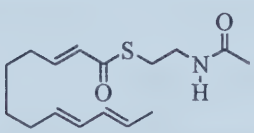
The triene thioester **80** of the compactin series can also be prepared and cyclized (Figure 16) by heating under reflux in toluene for 5 days. However the four decalins that are obtained, namely **104**, **105**, **106** and **107** could not be separated by GC-MS analysis.



(104) **(105)** **(106)** **(107)**

Figure 16. The thermal cyclized thioester products **104**, **105**, **106** and **107**.

2.2.7 Testing of dodeca-2,8,10-trienethoic acid *S*-(2-acetylaminoethyl) ester (78) with the *lovB* enzyme.

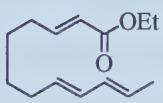


(78)

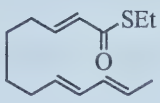
Four different concentrations of dodeca-2,8,10-trienethoic acid *S*-(2-acetylamino-ethyl) ester **78** were tested with the LovB enzyme. The reaction mixtures were extracted

and treated with a sodium ethoxide/ethanol solution. After work up the residues were dissolved in $CDCl_3$ and subjected to the developed GC-MS analysis method.

For all concentrations of material tested, four compounds corresponding to the ethyl esters **95**, **96**, **97** and **98** were observed eluting at retention times corresponding to those of the standards. The compounds are present in a 1 : 1 : 1 : 1 ratio for all concentrations tested, with no significant enhancement of the expected *endo* enzymatic compound, which would elute at 61.4 min (**95**). The results indicate that hexaketide **78**, as analogue of the intermediate on route to compactin, is not a good substrate for the LNKS enzyme.



(79)



(80)

2.2.8 Testing of dodeca-(*E*,*E*,*E*)-2,8,10-trienoic acid ethyl ester (79) and dodeca-(*E*,*E*,*E*)-2,8,10-trienethoic acid *S*-ethyl ester (80) with *lovB* enzyme.

The two ethyl esters, **79** and **80**, were tested with LovB using the same method as above. The respective mixtures that were obtained were dissolved in $CDCl_3$ and subjected to the GC-MS analysis. In both instances four compounds, corresponding to the ethyl esters **95**, **96**, **97**, and **98** were observed eluting in a 1 : 1 : 1 : 1 ratio, with no significant enhancement of the compound eluting at 61.4 min (**95**).

The results obtained indicate that none of the compactin intermediate analogues tested are good substrates for the LovB enzyme, however it is possible that some of these

compounds may be substrates for the enzyme obtained from the compactin gene sequence. This gene has not yet been cloned and/or overexpressed in a heterologous host, and is thus unavailable for testing (in contrast to the LovB enzyme used in these experiments). Some other interesting experiments that remains to be conducted involve incorporation studies where the chain length of the NAC ester **40** is varied, to determine if novel and perhaps more active analogues of lovastatin could be produced. X-ray studies on the LovB enzyme would also be useful to determine the properties of the active site, and to aid in the design of more suitable analogues. This might lead to the construction or cloning of a much smaller enzyme capable of accepting a variety of different substrates for Diels-Alder reactions. This has potential for opening up a new field in semisynthetic pharmaceuticals involving the enzymatic Diels-Alder reaction.

This project resulted in the synthesis of several possible substrates for testing the enzymatic Diels-Alder reaction, of which the NAC-ester **40** proved to be the only demonstrable substrate, [31] thereby in providing strong evidence that the Diels-Alder reaction is indeed part of the biosynthetic pathway in the formation of lovastatin. The synthesis of possible substrates for the testing of the compactin biosynthesis was also achieved, and suitable analytical techniques were developed to enable rapid testing of these and other new analogues.

3 Experimental

3.1 General

All reactions were performed under a positive pressure of dry argon (Ar), unless otherwise mentioned, using oven-dried glassware. All solvents were dried according to Perrin *et. al.*[32] Tetrahydrofuran (THF), diethyl ether, benzene and toluene were distilled over sodium under Ar atmosphere. Dichloromethane (CH_2Cl_2), dimethyl sulfoxide (DMSO), triethylamine (Et_3N) and pyridine were distilled from calcium hydride. Anhydrous ethanol and methanol were dried by distillation from magnesium turnings with a catalytic amount of iodine. Water was obtained from a Milli-Q reagent water system (Millipore Corp., Milford, MA). The removal of solvents from reaction mixtures was performed under reduced pressure using a Büchi rotary evaporator. Brine refers to a saturated sodium chloride solution. Unless otherwise specified, solutions of ammonium chloride, sodium hydrogen carbonate, potassium hydrogen carbonate, sodium carbonate, lithium hydroxide, potassium hydroxide and sodium hydroxide refer to aqueous solutions.

Air sensitive reagents were handled under an atmosphere of dry argon. All reagents employed were of the American Chemical Society (ACS) grade, or finer. All materials used in biological procedures were obtained from Sigma-Aldrich Canada, BDH Inc. (Toronto, Canada) or from Difco Laboratories (Detroit, MI). Electrophoresis gels and solutions were obtained from Bio Rad Canada (Mississauga, ON). All solutions, glassware and equipment used for fermentation were autoclaved at 121°C and 30 psi.

Where possible all reactions were followed by thin layer chromatography (TLC) and visualized employing UV fluorescence and/or phosphomolybdic acid/ceric sulfate/sulfuric acid (10.0 g : 1.25 g : 12 mL in 240.0 mL H_2O) staining solution.

Commercial silica TLC plates were supplied by Merck (60 F_{254}). Flash chromatography was performed according to Still *et. al.*[33], using Merck silica gel 60 (230-420 mesh). Preparative TLC plates were silica, from Merck (60 F_{254}, 1mm thickness). All eluants are listed as volume ratios.

Gas chromatography was performed on a HP5890 GC system using a HP MSD5970 mass spectrometer as detector. The column used was a β-DEX 120 from Supelco inc.

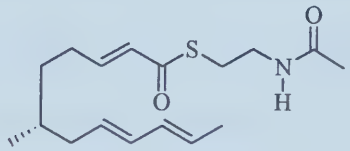
All literature compounds had infrared (IR), proton nuclear magnetic resonance (^{1}H NMR), and mass spectra (MS) consistent with reported data. Infrared spectra were recorded on a Nicolet Magna-IR 750 with Nic-Plan microscope FT-IR spectrometer. Cast refers to the evaporation of a solution on a sodium chloride plate to leave behind a cast of compound to be analyzed. MS were recorded on a Kratos AEI MS-50 (high resolution, electron impact ionization (EI)), a VG-70 (low resolution, chemical ionization (CI)), a HP 1100 (low resolution , electron spray ionization (ES)), and a ZabSpec Iso Mass VG (high resolution, ES).

NMR spectra were measured on a Varian-300 instrument. ^{1}H NMR chemical shifts are reported in parts per million (ppm) to the solvent resonance as the reference: $CDCl_3$ δ7.24, CD_3OD δ3.30, C_6D_6 δ7.15. Correlated spectroscopy (COSY) was occasionally used for signal assignments. ^{1}H NMR data is tabulated in the following order:

multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad), number of protons, coupling constant (s) in Hertz, and assignment.

Reference standards **43**, **45** and **47** were previously synthesized in our group by Dr. David J. Witter.

3.2 Synthesis of target compounds.



3.2.1 (6*R*)-(*E,E,E*)-6-methyldodeca-2,8,10-trienoic acid *N*-acetylcysteamine thioester (40).

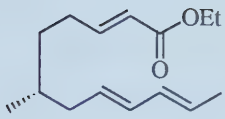
3.2.1.1 Method using acid (69) with 1,3-dicyclohexylcarbodiimide coupling.

A modification of the method by Parker[34] was used. (6*R*)-(*E,E,E*)-6-methyldodeca-2,8,10-trienoic acid **69** (215 mg, 1.03 mmol) was dissolved in CH_2Cl_2 (4.0 mL) and cooled to –15 °C. This solution was the treated simultaneously over 5 min. with a solution of *N*-acetylcysteamine **70** (145 mg, 1.22 mmol) in CH_2Cl_2 (2.0 mL), and a solution of 1,3-dicyclohexylcarbodiimide (255 mg, 1.24 mmol) in CH_2Cl_2 (2.0 mL). The mixture gradually turned cloudy, and was stirred overnight at room temperature. The mixture was then concentrated *in vacuo*, and the residue purified by preparative TLC (SiO_2, 100% EtOAc, R_f 0.33) to yield thioester **40** as a white solid (95 mg, 30%); IR

($CHCl_3$ cast) 3321, 3085, 3080, 2944, 2927, 2870, 2850, 1657, 1626 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.90 (dt, 1H, *J*=15.5, 7.4 Hz, H-3), 6.11 (dt, 1H, *J*=15.5, 1.5 Hz, H-2), 6.01 (ddq, 1H, *J*=14.2, 10.2, 1.5 Hz, H-10), 5.95 (ddt, 1H, *J*=14.2, 10.2, 1.2 Hz, H-9), 5.57 (dq, 1H, *J*=14.2, 6.6 Hz, H-11), 5.91-5.83 (br s, 1H, H-4'), 5.48 (dt, 1H, *J*=14.2, 7.2 Hz, H-8), 3.44 (dt, 2H, *J*=6.3, 5.9 Hz, H-3'), 3.07 (t, 2H, *J*=6.3 Hz, H-2'), 2.31-2.12 (m, 2H, H-4), 2.04 (ddd, 1H, *J*=13.7, 7.2, 6.1 Hz, 1 x H-7), 1.94 (ddd, 1H, *J*=13.7, 7.4, 7.2 Hz, 1 x H-7), 1.95 (s, 3H, H-6'), 1.72 (d, 3H, *J*=6.6 Hz, H-12), 1.55-1.45 (m, 2H, 1 x H-5, H-6), 1.30-1.25 (m, 1H, 1 x H-5), 0.87 (d, 3H, *J*=6.6 Hz, 6-CH_3); HRMS (ES) calcd for $C_{17}H_{27}NO_2S$ 309.1762, found 309.1756 $[M-1]^+$.

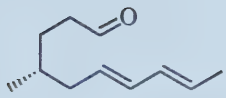
3.2.1.2 Method using Wittig reagent (71) and aldehyde (50).

The Wittig reagent **71** (0.88 g, 2.08 mmol) was dissolved in CH_2Cl_2 (5.0 mL) and treated with LiBr (0.18 g, 2.10 mmol) at room temperature. The mixture was stirred for 10 min. and then cooled to 0 °C. To this cooled solution was added dropwise triethylamine (212 mg, 300 μL, 2.10 mmol), and the mixture stirred for a further 20 min. at 0 °C. (4*R*)-(*E,E*)-4-Methyldeca-6,8-dienal **50** (0.35 g, 2.10 mmol) in CH_2Cl_2 (2.0 mL) was slowly added, and the mixture stirred at 0 °C for 30 min, and then at room temperature for 4 h. The mixture was concentrated *in vacuo* and the residue purified by preparative TLC (SiO_2, 100% EtOAc, R_f 0.33) to yield a white solid **40** (0.52 g, 1.61 mmol, 77%). The spectroscopic data for this solid was identical to that described for the above prepared NAC-ester.



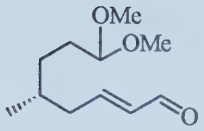
3.2.2 Ethyl-(6*R*)-(*E,E,E*)-6-methyldodeca-(2,8,10)-trienoate (41).

The method by Seebach and coworkers[39] was used. A solution of (4*R*)-4-methyldeca-(*E,E*)-6,8-dienal **50** (1.25 g, 7.54 mmol) and (carbethoxymethylene) triphenylphosphorane **58** (3.15 g, 9.04 mmol) in toluene (100 mL) was heated overnight under reflux. The solvent was removed *in vacuo*, and the residue purified by flash chromatography (SiO_2, 5% Et_2O in pentane, R_f 0.41) to yield a colourless oil **41** (1.11 g, 62%); IR ($CHCl_3$ cast) 3016, 2957, 2927, 2914, 2872, 2853, 1722, 1655 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.95 (dt, 1H, *J*=15.6, 6.9 Hz, H-3), 6.02 (ddq, 1H, *J*=14.2, 10.4, 1.5 Hz, H-10), 5.97 (ddt, 1H, *J*=14.4, 10.4, 1.3 Hz, H-9), 5.81 (dt, 1H, *J*=15.6, 1.6 Hz, H-2), 5.58 (dq, 1H, *J*=14.2, 6.9 Hz, H-11), 5.50 (dt, 1H, *J*=14.4, 7.3 Hz, H-8), 4.17 (q, 2H, *J*=6.5 Hz, H-1'), 2.29-2.10 (m, 2H, H-4), 2.06 (ddd, 1H, *J*=13.5, 7.3, 6.1 Hz, 1 x H-7), 1.93 (ddd, 1H, *J*=13.5, 7.3, 7.0 Hz, 1 x H-7), 1.73 (d, 3H, *J*=6.9 Hz, H-12), 1.56-1.43 (m, 2H, 1x H-5, 1 x H-6), 1.28-1.19 (m, 1H, 1 x H-5), 1.28 (t, 3H, *J*=6.5 Hz, H-2'), 0.90 (d, 3H, *J*=6.4 Hz, 6-CH_3), HRMS (ES)), calcd for $C_{15}H_{24}O_2$ 236.1776, found 236.1776 $[M]^+$.



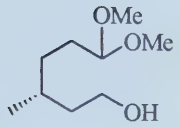
3.2.3 (4*R*)-(*E,E*)-4-Methyldeca-6,8-dienal (50).

A modified procedure of Roush and Hall[35] was used. (7*R*)-(*E,E*)-10,10-dimethoxy-7-methyldeca-2,4-diene **62** (1.07 g, 5.02 mmol) was dissolved in THF (20 mL), and treated with saturated aqueous oxalic acid (15 mL) at room temperature. The mixture was stirred at room temperature for 12 h, and then a further portion of saturated aqueous oxalic acid (2 mL) was added and stirring continued for 2 h. The mixture was partitioned between Et_2O (200 mL) and H_2O (50 mL), and the aqueous layer extracted with Et_2O (3 x 50 mL). The combined etheral extracts were washed with 5% aqueous $NaHCO_3$ (1 x 50 mL), H_2O (1 x 50 mL), and dried ($MgSO_4$). Concentration *in vacuo* gave a volatile yellow oil, which was purified by flash chromatography (SiO_2, 10% Et_2O in hexane, R_f 0.36) to yield aldehyde **50** as a colourless oil (0.70 g, 92%); IR ($CHCl_3$ cast) 3015, 2954, 2725, 1720 cm^{-1}; ^1H NMR (300 MHz, C_6D_6) δ 9.30 (t, 3H, *J*=1.7 Hz, H-1), 6.10-5.95 (m, 2H, H-7, H-8), 5.49 (dq, 2H, *J*=14.2, 1.7 Hz H-6), 5.38 (dt, 1H, *J*=14.1, 7.0 Hz, H-9), 1.95-1.67 (m, 4H, H-2, H-5), 1.58 (d, 3H, *J*=7.0 Hz, H-10), 1.44-1.35 (m, 1H, 1 x H-3), 1.25-1.15 (m, 1H, 1 x H-4), 1.15-1.07 (m, 1H, 1 x H-3), 0.67 (d, 3H, *J*=6.7 Hz, 4-CH_3); HRMS (ES) calcd for $C_{11}H_{18}O$ 166.1358, found 167.1072 $[M+1]^+$.



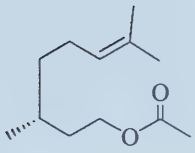
3.2.4 (5*R*)-(*E*)-8,8-Dimethoxy-5-methyloct-2-enal (51).

(5*R*)-(*E*)-8,8-Dimethoxy-5-methyloct-2-enol **60** (1.40 g, 6.93 mmol) was dissolved in CH_2Cl_2 (50 mL) and slowly added to a stirred solution of Dess-Martin reagent **56** (3.00 g, 6.9 mmol) in CH_2Cl_2 (30 mL) at room temperature. After 30 min. of stirring the mixture was diluted with Et_2O (100 mL), and aqueous NaOH (1.3 M, 80 mL) was added. The resulting mixture was stirred for a further 30min. at room temperature, and the organic layers separated. The organic extract was washed with aqueous NaOH (1.3 M, 1 x 50 mL) and H_2O (1 x 50 mL), and then dried over ($MgSO_4$). The solvent was removed *in vacuo* to yield an oily residue, which was further purified by flash chromatography (SiO_2, 50% Et_2O in pentane, R_f 0.35) to produce aldehyde **51** a colourless oil (0.98 g, 71%); IR ($CHCl_3$ cast) 2954, 2932, 2725, 2880, 1693, 1636, cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.30 (d, 1H, *J*=7.7 Hz, H-1), 6.09 (dt, 1H, *J*=15.5, 7.7 Hz, H-3), 5.90 (ddt, 1H, *J*=15.5, 7.7, 1.0 Hz, H-2), 4.21 (t, 1H, *J*=5.5 Hz, H-8), 3.14 (s, 6H, 2 x OCH_3), 1.76 (ddd, 1H, *J*=13.4, 7.7, 5.8 Hz, 1 x H-4), 1.59 (ddd, 1H, *J*=13.4, 7.7, 7.4 Hz, 1 x H-4), 1.56-1.40 (m, 2H, H-7), 1.28-1.15 (m, 2H, H-5, 1 x H-6), 1.10-0.99 (m, 1H, 1 x H-6), 0.61 (d, 3H, *J*=6.3 Hz, 5-CH_3); HRMS (ES) calcd for $C_{11}H_{20}O_3$ 200.1412, found 200.1412 $[M]^+$.



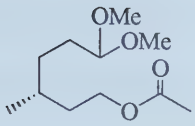
3.2.5 (3*R*)-6,6-Dimethoxy-3-methylhexanol (52).

To a solution of (3*R*)-6,6-dimethoxy-3-methylhexyl acetate **55** (3.0 g, 13.76 mmol) in dry MeOH (50 mL) was added sodium methoxide (80 mg, 1.5 mmol), and the resulting mixture stirred under argon at room temperature for 12 h. The reaction mixture was then treated with water (30 mL), and stirred for 15 min. The solvent was then removed under reduced pressure and the residue extracted with Et_2O (3 x 100 mL). The etheral extracts were washed with H_2O (2 x 50 mL), brine (1 x 50 mL), and dried over $MgSO_4$. Concentration *in vacuo* afforded an oil which was purified by flash chromatography (SiO_2, 20% EtOAc in hexane, R_f 0.10) to yield the alcohol **52** as a colourless oil (2.35 g, 97%) with spectroscopic properties consistent with reported data;[1] IR ($CHCl_3$ cast) 3409, 2953, 2929, 2873, 1129, 1509 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.31 (t, 1H, *J*=5.7 Hz, H-6), 3.66 (m, 2H, H-1), 3.28 (s, 6H, 2 x OCH_3), 1.52 (m, 4H, 1 x H-2, H-3, H-5), 1.34 (m, 1H, 1 x H-2), 1.18 (m, 2H, H-4), 0.88 (d, 3H, *J*=6.5 Hz, 3-CH_3); HRMS (ES) calcd for $C_9H_{20}O_3$ 176.1412, found 175.1338, $[M-1]^+$.



3.2.6 (*R*)-Citronellyl acetate (54).

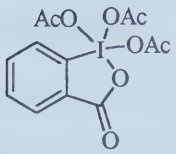
Acetyl chloride (2.72 mL, 38.4 mmol) was slowly added over 10 min. to an ice-cooled solution of (*R*)-citronellol **53** (4.38 g, 28.0 mmol) in THF (120 mL). The solution was stirred for 20 min. under argon at 0 °C and then treated with triethylamine (5.8 mL, 41.6 mmol) over a 60 min. period. The reaction mixture was then allowed to warm up to room temperature and stirred for a further 4 h under argon. Most of the solvent was then removed *in vacuo*, and the residue obtained was purified by flash chromatography (SiO_2, 5% EtOAc in hexane, R_f 0.32) to yield a colourless oil **54** (5.27 g, 95%) which had identical spectroscopic properties to that reported by Stork *et. al.*;[36] IR ($CHCl_3$ cast) 2966, 2918, 2873, 2859, 1743 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.07 (br t, 1H, *J*=7.0 Hz, H-6), 4.15-4.05 (m, 2H, H-1), 2.03 (s, 3H, H-2'), 2.05-1.92 (m, 2H, H-5), 1.70 (m, 1H, H-2), 1.67 (s, 3H, HC=C$\underline{CH_3}$), 1.59 (s, 3H, HC=C$\underline{CH_3}$), 1.58 (m, 1H, H-3), 1.48-1.38 (m, 1H, H-2), 1.38 (m, 1H, H-4), 1.28 (m, 1H, H-4), 0.90 (d, 3H, *J*=6.6 Hz, 3-CH_3); HRMS (ES) calcd. for $C_{12}H_{22}O_2$ 198.1620, found 198.1624, $[M]^+$.



3.2.7 (3*R*)-6,6-Dimethoxy-3-methylhexyl acetate (55).

The method of Claus and Schreiber[37] was used. A 3-necked round bottom flask was fitted with a drying tube ($CaCl_2$), a glass tube used to bubble the ozone through, and a glass stopper. A solution of (*R*)-citronellyl acetate **54** (5.0 g, 25.2 mmol) in methanol (200 mL) was cooled to –78 °C under argon, and stirred for 5 min. Ozone was then bubbled through the solution, until a light blue colour persisted. The excess ozone was purged by bubbling argon through the reaction mixture for 30 min. *p*-Toluenesulfonic acid (*p*-TsOH) was then added (500 mg, 10% by weight) and the solution was allowed to warm to room temperature and stirred for an additional 2 hours. Dimethylsulfide (5.0 mL, 55.0 mmol) was then slowly added and the solution stirred overnight under argon. Most of the solvent was then removed *in vacuo* and CH_2Cl_2 (100 mL) added. The mixture was washed with H_2O (1 x 100 mL), and the aqueous layer extracted with CH_2Cl_2 (2 x 100 mL). The combined CH_2Cl_2 extracts were washed with water (1x100 mL), dried over magnesium sulfate ($MgSO_4$) and concentrated *in vacuo* to yield a crude oil. This was purified by flash chromatography (SiO_2, 20% EtOAc in hexane, R_f 0.25) to yield acetate **55** as a colourless oil (3.23 g, 59%), exhibiting identical spectroscopic properties to previously reported data;[1h] IR ($CHCl_3$ cast) 3409, 2953, 2929, 2873, 1129, 1509 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.30 (t, 1H, *J*=5.7 Hz, H-6), 4.12-4.00 (m, 2H, H-1), 3.28 (s,

6H, 2 x OCH_3), 1.99 (s, 3H, H-2'), 1.60 (m, 4H, 1 x H-2, H-3, H-5), 1.40 (m, 2H, 1 x H-2, 1xH-4), 1.28 (m, 1H, 1 x H-4), 0.90 (d, 3H, J=6.5 Hz, 3-CH_3); HRMS (ES) calcd for $C_{11}H_{22}O_4$ 218.1518, found 187.1333, $[M\text{-}OCH_3]^+$.



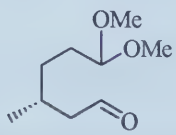
3.2.8 Dess-Martin periodinane (56).

3.2.8.1 Preparation employing original method by Dess and Martin.[27]

2-Iodobenzoic acid (21.3 g, 85 mmol) was dissolved in aqueous sulfuric acid (0.73 M, 400 mL) and stirred at room temperature forming a white suspension. Potassium bromate (19.0 g, 112.5 mmol) was then slowly added, taking care to keep the temperature below 55 °C. The addition of $KBrO_3$ took approximately 45 min. The suspension was then stirred for 4 h at 65 °C, and was then cooled to 0 °C for 1 h. The solid that formed was quickly filtered off, and washed with H_2O (2 x 100 mL) and ethanol (2 x 50 mL). The washed solid was then taken up in a mixture of acetic anhydride (60 mL) and acetic acid (60 mL). This mixture was heated to 100 °C until the solid had just dissolved, and then left to cool overnight at 4 °C. The white powder that formed was then removed by filtration and washed with Et_2O (2 x 50 mL), to yield the desired product **56** (25.90 g, 71%), which was used without any further purification; ^{1}H NMR (300 MHz, CD_3OD) δ 8.15 (m, 1H, Ar-H), 7.83 (m, 2H, Ar-H), 7.70 (m, 1H, Ar-H), 2.05 (s, 9H, 3 x CH_3).

3.2.8.2 Preparation using oxone.[28]

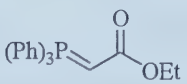
A combination of the methods by Frigerio and coworkers, as well as by Ireland and Liu[28] was used. 2-Iodobenzoic acid (12.0 g, 48.4 mmol) was suspended in H_2O (145 mL) containing oxone (38.6 g, 62.7 mmol), and stirred at 70 °C for 3 h. The suspension was cooled to 0 °C for 1 h, and the solid filtered off and washed with H_2O (3 x 50 mL) and acetone (2 x 50 mL). The solid was transferred to a stirred solution of acetic anhydride (50 mL) containing a catalytic amount of *p*-Toluenesulfonic acid (60 mg). This suspension was heated under argon to 80 °C for 3 h, and was then cooled to 4 °C overnight. The white powder that formed was filtered off, and washed with Et_2O (2 x 50 mL), to yield the desired product **56** (18.30 g, 59%), which was used without any further purification. ^1H NMR data was identical to that of the product using the original method.



3.2.9 (3*R*)-6,6-Dimethoxy-3-methylhexanal (57).

(3*R*)-6,6-Dimethoxy-3-methylhexanol **52** (1.50 g, 8.52 mmol) was dissolved in CH_2Cl_2 (50 mL) and slowly added to a stirred solution of freshly prepared Dess-Martin periodinane **56** (4.00 g, 9.30 mmol) in CH_2Cl_2 (30 mL) at room temperature. After 30 min. the mixture was diluted with Et_2O (100 mL), and aqueous NaOH (1.3 M, 80 mL) was added. The resulting mixture was stirred for a further 30 min. at room temperature, and the organic layer separated. The organic extract was washed with aqueous NaOH

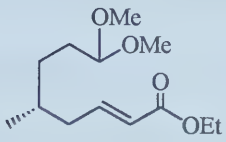
(1.3 M, 1 x 50 mL), H_2O (1 x 50 mL), and then dried ($MgSO_4$). Concentration under reduced pressure yielded an oily residue, which was further purified by flash chromatography (SiO_2, 50% Et_2O in pentane, R_f 0.91) to afford aldehyde **57** as a colourless oil (1.16 g, 78%), exhibiting identical spectroscopic properties to previously reported data;[1] IR ($CHCl_3$ cast) 2955, 2953, 2935, 2880, 2831, 2720, 1725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.71 (t, 1H, *J*=2.2 Hz, H-1), 4.30 (t, 1H, *J*=5.6 Hz, H-6), 3.27 (s, 6H, 2 x OCH_3), 2.38 (ddd, 1H, *J*=16.0, 5.8, 2.2 Hz, 1xH-2), 2.21 (ddd, 1H, *J*=16.0, 7.8, 2.2 Hz, 1 x H-2), 2.04 (m, 1H, H-3), 1.71-1.48 (m, 2H, 1 x H-5), 1.41-1.12 (m, 2H, H-4), 0.93 (d, 3H, *J*=6.7 Hz, 3-CH_3); HRMS (ES) calcd for $C_9H_{18}O_3$ 174.1256, found 173.1178 $[M-1]^+$.



3.2.10 (Carbethoxymethylene)triphenylphosphorane (58).

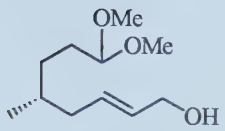
The method of Isler *et. al.*[38] was used. Triphenylphosphine (13.8 g, 52.5 mmol) was dissolved in toluene (50 mL) and treated with ethyl-2-bromoacetate (7.7 g, 45.8 mmol) at room temperature. The mixture was then heated under reflux at 110 °C for 1 h, and cooled to room temperature. The white powder that formed was removed by filtration, and washed with hexane (2 x 50 mL). This salt could be stored at room temperature until needed. When required, the salt was dissolved in H_2O (200 mL) and CH_2Cl_2 (200 mL) was added. This biphasic mixture was then cooled to 0 °C and treated with aqueous NaOH (2.2 g in 25 mL H_2O) over a 1 h period. The mixture was stirred for a further 30 min. at 0 °C, and then the CH_2Cl_2 layer was separated, dried ($MgSO_4$), and concentrated

in vacuo to yield the desired compound **58** (13.5 g, 38.8, 73%), which was used without any further purification; mp 128 °C (lit.[38] mp 128-130 °C) MS (EI) calcd for $C_{22}H_{21}O_2P$ 348.1279, found 348.1199 $[M]^+$.



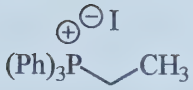
3.2.11 Ethyl-(5*R*)-(*E*)-8,8-dimethoxy-5-methyloct-2-enoate (59).

A modification of the method reported by Seebach and coworkers[39] was used. A solution of (3*R*)-6,6-dimethoxy-3-methylhexanal **57** (1.0 g, 5.75 mmol) and (carbethoxymethylene) triphenylphosphorane **58** (2.50 g, 7.18 mmol) in toluene (50 mL) was heated under reflux overnight at 85 °C. The solvent was removed *in vacuo* and the residue obtained was purified by flash chromatography (SiO_2, 15% Et_2O in pentane, R_f 0.14) to yield a colourless oil **59** (0.94 g, 67%); IR ($CHCl_3$ cast) 2956, 2953, 2931, 1772, 1654 cm^{-1}; ^1H NMR (300 MHz, C_6D_6) δ 7.03 (dt, 1H, *J*=15.5, 7.7 Hz, H-3), 5.87 (dt, 1H, *J*=15.5, 1.2 Hz, H-2), 4.19 (t, 1H, *J*=5.7 Hz, H-8), 4.04 (q, 2H, *J*=7.1 Hz, H-1'), 3.12 (s, 6H, 2 x OCH_3), 1.85 (dddd, 1H, *J*=13.0, 7.7, 5.3, 1.2 Hz, 1 x H-4), 1.67 (dddd, 1H, *J*=13.0, 7.7, 7.7, 1.2 Hz, 1 x H-4), 1.60-1.50 (m, 1H, 1 x H-7), 1.50-1.40 (m, 1H, 1 x H-7), 1.32-1.20 (m, 2H, H-5 & 1 x H-6), 1.11-1.02 (m, 1H, 1 x H-6), 0.98 (t, 3H, *J*=7.1 Hz, H-2'); 0.67 (d, 3H, *J*=6.6 Hz, 5-CH_3); HRMS (Es) calcd for $C_{13}H_{24}O_4$ 244.1675, found 243.1599 $[M-1]^+$.



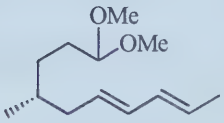
3.2.12 (5*R*)-(*E*)-8,8-Dimethoxy-5-methyloct-2-enol (60).

A modification of the method reported by Nicolau *et. al.*[40] was used. Ethyl (5*R*)-(*E*)-8,8-dimethoxy-5-methyloct-2-enoate **59** (2.0 g, 8.20 mmol) was dissolved in CH_2Cl_2 (50 mL) and cooled to –78 °C. Diisobutylaluminum hydride (1.0 M in CH_2Cl_2, 18.0 mL, 18.0 mmol) was then added to the above solution over a 15 min. period, and stirred for a further 2 h at –78 °C. The reaction was quenched by addition of saturated aqueous NH_4Cl (10 mL), and allowed to warm to room temperature. The reaction mixture was diluted with Et_2O (200 mL) and filtered through celite. The filtrate was dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the crude product by flash chromatography (SiO_2, 75% Et_2O in pentane, R_f 0.31) afforded a colourless oil **60** (1.40 g, 84%) with identical spectroscopic properties to reported data;[1h] IR ($CHCl_3$ cast) 3420, 2952, 2930, 1458, 1384 cm^{-1}; ^1H NMR (300 MHz, C_6D_6) δ 5.55-5.49 (m, 2H, H-2, H-3), 5.87 (dt, 1H, *J*=15.5, 1.2 Hz, H-2), 4.27 (t, 1H, *J*=15.5, 7.7 Hz, H-3), 4.19 (t, 1H, *J*=5.7 Hz, H-8), 4.04 (q, 1H, *J*=5.6 Hz, H-8), 3.91 (br s, 2H, H-1), 3.14 (s, 6H, 2 x OCH_3), 1.99-1.90 (m, 1H, 1 x H-4), 1.84-1.75 (m, 1H, 1 x H-4), 1.70-1.50 (m, 3H, OH, H-7), 1.45-1.33 (m, 2H, H-5, 1 x H-6), 1.22-1.12 (m, 1H, 1 x H-6), 0.82 (d, 3H, *J*=6.5 Hz, 5-CH_3); HRMS (ES) calcd for $C_{11}H_{22}O_3$ 202.1569, found 202.1567 $[M]^+$.



3.2.13 Ethyltriphenylphosphonium iodide (61).

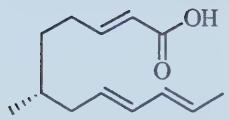
The method of Barnhardt and McEwan[41] was used. Triphenylphosphine (6.0 g, 22.9 mmol) was dissolved in toluene (30 mL), and treated with iodoethane (1.75 mL, 21.8 mmol) at room temperature. The mixture was then heated under reflux at 110 °C for 1 h, and then cooled to room temperature. The crystalline material was filtered to afford **61** (7.27 g, 80%) which was used without any further purification; mp 158 °C (lit.[41] mp 164-165 °C).



3.2.14 (7*R*)-(*E*,*E*)-10,10-Dimethoxy-7-methyldeca-2,4-diene (62).

A modification of the method of Schlosser and Christmann[42] was used. Ethyltriphenylphosphonium iodide **61** (1.88 g, 4.5 mmol) was suspended in THF (20 mL) and Et_2O (20 mL). *n*-Butyllithium (2.5 M in hexane, 2.0 mL, 5.0 mmol) was added to the above suspension and the mixture stirred for 15 min. at room temperature. The reaction mixture turned bright red in colour and was then cooled to –78 °C. The (5*R*)-(*E*)-8,8-dimethoxy-5-methyloct-2-enal **51** (0.90 g, 4.5 mmol) in Et_2O (5.0 mL) was slowly added,

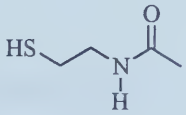
and stirring continued for 15 min. A second portion of *n*-butyllithium (2.5 M in hexane, 2.0 mL, 5.0 mmol) was added, and stirring continued for a further 5 min. Etheral HCl (1.0 M, 5.0 mL, 5.0 mmol) was added, followed by addition of potassium-*t*-butoxide (0.90 g, 10.0 mmol) in *t*-BuOH (10.0 mL). The mixture was then stirred for 2 h at room temperature, and then it was diluted with Et_2O (200 mL). The organic layer was then washed with H_2O (4 x 100 mL) and brine (1 x 100 mL), where after it was dried ($MgSO_4$) and concentrated *in vacuo* to yield a yellow oil, which was further purified by flash chromatography (SiO_2, 5% Et_2O in pentane, R_f 0.30) to yield diene **62** as an oil (0.85 g, 88%); IR ($CHCl_3$ cast) 3016, 2952, 2929, 1457, 1378 cm^{-1}; ^1H NMR (300 MHz, C_6D_6) δ 6.12-5.99 (m, 2H, H-3, H-4), 5.53-5.43 (m, 2H, H-2, H-5), 4.28 (t, 1H, *J*=5.7 Hz, H-10), 3.15 (s, 6H, 2 x OCH_3), 2.04 (ddd, 1H, *J*=13.8, 7.0, 7.0 Hz, 1 x H-6), 1.87 (ddd, 1H, *J*=13.8, 7.0, 6.5 Hz, 1 x H-6), 1.72-1.52 (m, 2H, H-9), 1.62 (d, 3H, *J*=6.7 Hz, H-1), 1.48-1.38 (m, 2H, H-7, 1 x H-8), 1.27-1.18 (m, 1H, 1 x H-8), 0.83 (d, 3H, *J*=6.6 Hz, 7-CH_3); HRMS (ES) calcd for $C_{13}H_{24}O_2$ 212.1776, found 212.1776 $[M]^+$.



3.2.15 (6*R*)-(*E,E,E*)-6-Methyldodeca-2,8,10-trienoic acid (69).

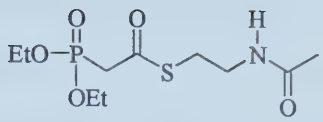
The procedure by Seebach and coworkers[39] was employed. Ethyl (6*R*)-(*E,E,E*)-6-methyldodeca-2,8,10-trienoate **41** (500 mg, 2.11 mmol) was dissolved in THF (30 mL), and treated with aqueous LiOH·1 H_2O (3.0 M, 5.0 mL). The mixture was stirred at 60 °C for 12 h, and most of the solvent was removed *in vacuo*. The residue was again heated to 60 °C until it turned from cloudy to clear, and was cooled to room temperature. This mixture was diluted with H_2O (250 mL), and washed with pentane (2 x 30 mL). The aqueous fraction was acidified to pH 7.0 using 1.0 M HCl, and then extracted with Et_2O (3 x 100 mL). The combined Et_2O extracts were dried ($MgSO_4$) and concentrated in vacuo to yield an oil, which was purified by flash chromatography (SiO_2, 100% Et_2O, R_f 0.50) to yield acid **69** (218 mg, 42%); IR ($CHCl_3$ cast) 2958, 2914, 1696, 1650 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 11.20 (br s, 1H, COOH), 7.08 (dt, 1H, *J*=15.6, 6.9 Hz, H-3), 6.02 (ddq, 1H, *J*=14.2, 10.4, 1.5 Hz, H-10), 5.98 (ddt, 1H, *J*=14.4, 10.4, 1.3 Hz, H-9), 5.82 (dt, 1H, *J*=15.6, 1.6 Hz, H-2), 5.58 (dq, 1H, *J*=14.2, 6.9 Hz, H-11), 5.50 (dt, 1H, *J*=14.4, 7.3 Hz, H-8), 2.34-2.14 (m, 2H, H-4), 2.05 (ddd, 1H, *J*=13.8, 7.3, 6.4 Hz, 1 x H-7), 1.93 (ddd, 1H, *J*=13.8, 7.3, 7.0 Hz, 1 x H-7), 1.73 (d, 3H, *J*=6.9 Hz, H-12), 1.57-1.45

(m, 2H, 1xH-5, H-6), 1.32-1.22 (m, 1H, 1 x H-5), 0.88 (d, 3H, J=6.6 Hz, 6-CH_3); HRMS (ES) calcd for $C_{13}H_{20}O_2$ 208.1463, found 208.1461 $[M-1]^+$.



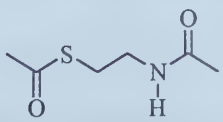
3.2.16 *N*-Acetylcysteamine (70).

A modification of the method by Schwab and Klassen[43] was used. *N,S*-Diacetyl-*β*-mercaptoethylamine **72** (30.40 g, 189 mmol) was dissolved in H_2O (600 mL) and cooled to 0 °C. This solution was then treated with solid KOH (33.8 g, 189 mmol) over 30 min. The resulting mixture was stirred under argon at room temperature for an additional 2 h, and then acidified to pH 7.0 with 2.0 M HCl. The mixture was then saturated with NaCl and extracted with CH_2Cl_2 (5 x 200 mL). The organic fractions were combined, dried ($MgSO_4$), and concentrated *in vacuo* to yield cysteamine **70** (22.7 g, 99%) which could be used without any further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (br s, 1H, NH), 3.43 (dt, 2H, J=6.4, 5.9 Hz, CH_2NH), 2.68 (ddt, 2H, J=8.3, 6.4, 1.9 Hz, SCH_2), 2.02 (s, 3H, $COCH_3$), 1.36 (t, 1H, J=8.3 Hz, SH); HRMS (ES) calcd for C_4H_9NOS 119.0405, found 119.0405 $[M]^+$.



3.2.17 (Diethoxyphosphoryl)thioacetic acid *S*-(2-acetylamino-ethyl) ester (71).

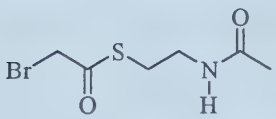
The *N*-acetyl-*S*-(2-bromoacetyl)-cysteamine thioester **74** (600 mg, 2.34 mmol) was dissolved in toluene (20 mL) and treated with triethylphosphite (428 mg, 0.44 mL, 2.58 mmol). The mixture was then heated under reflux for 48 h, and the solvent removed *in vacuo* to yield a yellow oil **71** (880 mg, 89%) which was used without any further purification; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.25 (br s, 1H, NH), 4.14 (q, 4H, *J*=7.3 Hz, OCH_2CH_3), 3.41 (q, 2H, *J*=6.2 Hz, CH_2N), 3.19 (d, 2H, *J*=21.3 Hz, CH_2PO), 3.04 (t, 2H, *J*=6.3 Hz, SCH_2), 1.86 (s, 3H, CH_3CO), 1.13 (t, 6H, *J*=7.0 Hz, OCH_2CH_3); HRMS (ES) calcd for $C_{10}H_{20}NO_5PS$ 297.0800, found 269.0850 $[M\text{-}CO]^+$.



3.2.18 *N,S*-Diacetyl-*β*-mercaptoethylamine (73).

The procedure of Gerstein and Jencks[44] was used. A solution of 2-mercaptoethylamine hydrochloride (56.8 g, 500 mmol) in H_2O (150 mL) was cooled to –5 °C, and then simultaneously (over 90 min.) treated with acetic anhydride (142 mL, 1.5 mol) and

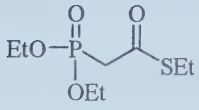
aqueous 8 M KOH, in such a manner that the pH of the solution was maintained at pH 8. The reaction mixture was then stirred for a further 60 min. at room temperature, and the product extracted with Et_2O (3 x 150 mL). The combined Et_2O extracts were dried ($MgSO_4$) and concentrated *in vacuo* to yield a colourless oil **73** (66.0 g, 82%) which could be used without any further purification; IR ($CHCl_3$ cast) 3251, 2940, 1734, 1667 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.40 (br s, 1H, NH), 3.40-3.36 (m, 2H, CH_2NH), 3.02 (t, 2H, J=6.4 Hz, SCH_2), 2.33 (s, 3H, CH_3COS), 1.97 (s, 3H, $NHCOCH_3$); HRMS (ES) calcd for $C_6H_{11}NO_2S$ 161.0510, found 161.0510 $[M]^+$.



3.2.19 *N*-Acetyl-*S*-(2-bromoacetyl)cysteamine thioester (74).

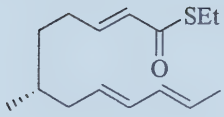
The method of Roblot and Wylde[45] was employed. To a solution of *N*-acetylcysteamine **70** (1.19 g, 10 mmol) in THF (15 mL) at room temperature was slowly (10 min) added bromoacetyl bromide (2.02 g, 0.87 mL, 10 mmol). The above mixture was stirred for 5 min. at room temperature, and then the reaction was quenched by addition of saturated aqueous $KHCO_3$ (10 mL). The product was extracted with CH_2Cl_2 (3 x 50 mL), and the combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo* to yield a brown oil. Purification by flash chromatography (SiO_2, 100% Et_2O, R_f 0.26) yielded a colourless oil **74** (0.96 g, 40%); IR ($CHCl_3$ cast) 3251, 2940, 1734, 1667 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.83-5.65 (br s, 1H, NH), 4.10 (s, 2H, $COCH_2Br$), 3.40 (t, 2H,

J=6.2 Hz, C$\underline{H}_2$N), 3.10 (t, 2H, *J*=6.41 Hz, C$\underline{H}_2$SCO), 2.00 (s, 3H, CH_3CO); HRMS (ES) calcd for $C_6H_{10}O_2NSBr$ 238.9616, found 238.9620 $[M]^+$.



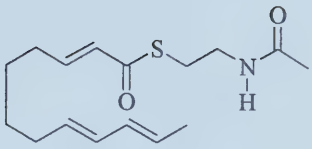
3.2.20 (Diethoxyphosphoryl)thioacetic acid *S* ethyl ester (76).

Ethanethiol (3.57 g, 4.26 mL, 57.39 mmol) in THF (20.0 mL) was slowly (over 10 min.) added to a solution of bromoacetylbromide (11.58 g, 5.0 mL, 57.39 mmol) in THF (30.0 mL) at 0 °C. The mixture was stirred at 0 °C for a further 10 min., and then the reaction was quenched by addition of saturated aqueous $KHCO_3$ (20.0 mL). The mixture was extracted with CH_2Cl_2 (3 x 50 mL), and the combined CH_2Cl_2 extracts were dried ($MgSO_4$) and concentrated *in vacuo* to a yellow oil (9.15 g, 87%). This oil, which was used without any further purification, was heated under reflux for 48 hours with triethylphosphite (9.55 g, 9.69 mL, 57.39 mmol) in benzene (100 mL). The solvent was then removed in vacuo to yield a red oil **76** (10.32 g, 86%), which could be used without any further purification; IR ($CHCl_3$ cast) 2981, 2932, 1736, 1684, 1606, 1478 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.13 (m, 6H, -SC$\underline{H}_2$-, 2 x -OC$\underline{H}_2$$CH_3$), 3.19 (d, 2H, *J*=21.3 Hz, -POC$\underline{H}_2$-), 1.20-1.40 (m, 9H, -$SCH_2C\underline{H}_3$, 2 x -$OCH_2C\underline{H}_3$); HRMS (ES) calcd for $C_8H_{17}O_4PS$ 240.0585, found 240.0584 $[M]^+$.



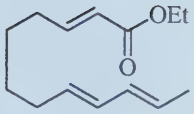
3.2.21 (6*R*)-Methyldodeca-(*E*,*E*,*E*)-2,8,10-trienethioic acid *S* ethyl ester (77).

The (diethoxyphosphoryl)thioacetic acid *S*-ethyl ester **76** (90 mg, 0.354 mmol) was dissolved in CH_2Cl_2 (5.0 mL) and treated with LiBr (30 mg, 0.354 mmol) at room temperature. The mixture was stirred for 10 min., and then cooled to 0 °C. This cooled solution was then treated with triethylamine (48 mg, 60 μL, 0.48 mmol), and stirred for 20 min. at 0 °C. (4*R*)-(*E*,*E*)-4-Methyldeca-6,8-dienal **50** (55 mg, 0.234 mmol) in CH_2Cl_2 (2.0 mL) was slowly added, and the mixture stirred at 0 °C for 30 min, and then at room temperature for 4 h. The mixture was concentrated *in vacuo* and the residue obtained was purified by preparative TLC (SiO_2, 100% EtOAc, R_f 0.33) to yield a red oil **77** (50 mg, 61%); IR ($CHCl_3$ cast) 3085, 3080, 2944, 2927, 2870, 2850, 1657, 1626 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.86 (dt, 1H, *J*=15.5, 7.4 Hz, H-3), 6.08 (dt, 1H, *J*=15.5, 1.5 Hz, H-2), 5.98 (m, 2H, H-8, H-10), 5.50 (m, 2H, H-9, H-11), 2.85 (q, 2H, *J*=6.8 Hz, H-1'), 2.31-2.12 (m, 2H, H-4), 2.04 (m, 2H, H-7), 1.72 (d, 3H, *J*=6.6 Hz, H-12), 1.55-1.45 (m, 2H, 1 x H-5, H-6), 1.30-1.25 (m, 1H, 1 x H-5), 1.22 (t, 3H, *J*=6.8 Hz, H-2'), 0.87 (d, 3H, *J*=6.6 Hz, 6-CH_3); HRMS (ES) calcd for $C_{15}H_{24}OS$ 252.1548, found 252.1548 $[M]^+$.



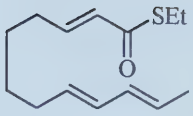
3.2.22 Dodeca-(*E*,*E*,*E*)-2,8,10-trienethoic acid *S*-(2-acetylamino-ethyl) ester (78).

The same method as for the preparation of *N*-acetylcysteamine thioester **40** was followed. The Wittig reagent **71** (880 mg, 2.08 mmol) was dissolved in CH_2Cl_2 (5.0 mL) and treated with LiBr (180mg, 2.10 mmol) at room temperature. The mixture was stirred for 10 min., and then cooled to 0 °C. This cooled solution was then slowly treated with triethylamine (212 mg, 300 μL, 2.10 mmol), and stirred for 20 min. at 0 °C. (*E*,*E*)-Deca-6,8-dienal **86** (347 mg, 2.10 mmol) in CH_2Cl_2 (2.0 mL) was slowly added, and the mixture stirred at 0 °C for 30 min, and then at room temperature for 4 h. The mixture was concentrated *in vacuo* and the residue was purified by preparative TLC (SiO_2, 100% EtOAc, R_f 0.35) to yield thioester **78** as a white solid (612 mg, 56%). IR ($CHCl_3$ cast) 3321, 3085, 3080, 2944, 2927, 2870, 2850, 1657, 1626 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.89 (dt, 1H, *J*=15.6, 6.9 Hz, H-3), 6.09 (dt, 1H, *J*=15.6, 3.0 Hz, H-2), 5.95 (m, 2H, H-8, H-9), 5.0 (br s, 1H, H-4'), 5.48 (m, 2H, H-10, H-11), 3.43 (dt, 2H, *J*=6.3, 5.9 Hz, H-3'), 3.07 (t, 2H, *J*=6.3 Hz, H-2'), 2.24-2.16 (m, 2H, H-4), 2.08-1.99 (m, 2H, H-7), 1.95 (s, 3H, H-6'), 1.70 (d, 3H, *J*=6.3 Hz, H-12), 1.50-1.35 (m, 4H, H-5, H-6); HRMS (ES) calcd for $C_{16}H_{25}NO_2S$ 295.1606, found 295.1606 $[M]^+$.



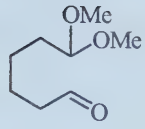
3.2.23 Dodeca-(*E*,*E*,*E*)-2,8,10-trienoic acid ethyl ester (79).

The method of Seebach and coworkers[39] was employed. A solution of (*E*,*E*)-deca-6,8-dienal **86** (500 mg, 3.29 mmol) and (carbethoxymethylene)triphenylphosphorane **58** (1.32 g, 3.8 mmol) in toluene (100 mL) was heated overnight under reflux. The solvent was removed *in vacuo*, and the residue purified by flash chromatography (SiO_2, 5% Et_2O in pentane, R_f0.40) to yield a colourless oil **79** (321 mg, 44%); IR ($CHCl_3$ cast) 3015, 2979, 1721, 1653 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.02 (dtd, 1H, *J*=15.7, 7.0, 1.6 Hz, H-3), 6.37 (ddq, 1H, *J*=14.9, 10.7, 1.6 Hz, H-10), 5.98 (m, 1H, H-9), 5.86 (ddt, 1H, *J*=16.4, 14.5, 1.6 Hz, H-2), 5.35 (m, 1H, H-11), 5.50 (m, 1H, H-8), 4.06 (q, 2H, *J*=7.1 Hz, H-1'), 1.87 (m, 2H, H-7), 1.77 (m, 2H, H-4), 1.62 (d, 3H, *J*=7.1 Hz, H-12), 1.30 (m, 2H, 1 x H-5, 1 x H-6), 1.15 (m, 2H, 1 x H-5, 1 x H-6), 1.00 (t, 3H, *J*=7.1 Hz, H-2'); HRMS (ES) calcd for $C_{14}H_{22}O_2$ 222.1620, found 222.1621 $[M-1]^+$.



3.2.24 Dodeca-(*E*,*E*,*E*)-2,8,10-trienethoic acid *S* ethyl ester (80).

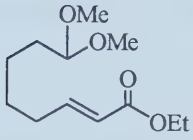
The same method as for the preparation of the thioester **77** was followed. The (diethoxy-phosphoryl)-thioacetic acid *S*-ethyl ester **76** (90 mg, 0.354 mmol) was dissolved in CH_2Cl_2 (5.0 mL) and treated with LiBr (30 mg, 0.354 mmol) at room temperature. The mixture was stirred for 10 min. and then cooled to 0 °C. Triethylamine (48 mg, 60 μL, 0.48 mmol) was slowly added, and stirring continued for 20 min. at 0 °C. Deca-(*E*,*E*)-6,8-dienal **86** (54 mg, 0.234 mmol) in CH_2Cl_2 (2.0 mL) was added, and the mixture stirred at 0 °C for 30 min, and then at room temperature for 4 h. The mixture was concentrated *in vacuo*, and the residue obtained purified by preparative TLC (SiO_2, 100% EtOAc, R_f0.33) to yield an oil **80** (52 mg, 62%); IR ($CHCl_3$ cast) 3015, 2979, 1721, 1653 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.85 (dt, 1H, *J*=15.5, 7.0 Hz, H-3), 6.65 (dt, 1H, *J*=6.9, 15.5 Hz, H-2), 5.99 (m, 1H, H-10), 5.52 (m, 1H, H-9), 2.92 (q, 2H, *J*=7.4 Hz, H-1'), 2.16 (m, 2H, H-7), 2.02 (m, 2H, H-4), 1.70 (d, 3H, *J*=6.3 Hz, H-12), 1.40 (m, 4H, H-5, H-6), 1.24 (t, 3H, *J*=7.4 Hz, H-2'); HRMS (ES) calcd for $C_{14}H_{22}OS$ 238.1391, found 283.1391 $[M]^+$.



3.2.25 6,6-Dimethoxyhexanal (81).

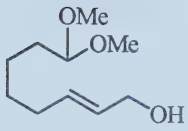
The method of Claus and Schreiber[37] was employed. A 500 mL 3-necked round bottom flask was fitted with a drying tube ($CaCl_2$), a glass tube used to bubble ozone through, and a glass stopper. The flask was charged with cyclohexene (6.16 g, 75 mmol) and dry MeOH (200 mL). The mixture was cooled to –78 °C under argon, and stirred for 5 min. Ozone was then bubbled through the solution, until a light blue colour persisted. The excess ozone was purged by bubbling argon through the solution for 30 min. (the solution became colourless). *p*-Toluenesulfonic acid (600 mg, 10% by weight) was then added, and the solution was allowed to warm to room temperature, and stirred for 2 h. Anhydrous $NaHCO_3$ (2.5 g, 26 mmol) was added to neutralize the *p*-Toluenesulfonic acid, and the mixture was stirred for 20 min at room temperature. Dimethylsulfide (12 mL, 150 mmol) was slowly added by syringe, and the solution stirred overnight under argon. Most of the solvent was then removed *in vacuo*, and a portion of CH_2Cl_2 (100 mL) added. The mixture was washed with H_2O (1 x 100 mL), and the water extracted with CH_2Cl_2 (2 x 100 mL). CH_2Cl_2 extracts were combined and washed with water (1 x 100 mL) and dried over $MgSO_4$. Concentration *in vacuo* yielded a crude oil, which was purified by flash chromatography (SiO_2, 20% EtOAc in hexane, R_f 0.25) to yield

aldehyde **81** as a colourless oil (11.20 g, 93%); IR ($CHCl_3$ cast) 2947, 2830, 2721, 1725, 1129, 1462 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.72 (t, 1H, *J*=2.5 Hz), 4.30 (t, 1H, *J*=5.3 Hz), 3.28 (s, 6H, 2 x OCH_3), 2.41 (t, 2H, *J*=7.0 Hz), 1.71-1.40 (m, 6H); HRMS (ES), calcd for $C_8H_{16}O_3$ 160.1099, found 159.1015, $[M-1]^+$.



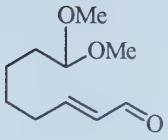
3.2.26 8,8-Dimethoxyoct-2-enoic acid ethyl ester (82).

A modification of the method by Schreiber *et. al.*[46] was used. A solution of 6,6-dimethoxyhexanal **81** (11.20 g, 57 mmol) and (carbethoxymethylene) triphenylphosphorane **58** (24.36 g, 70 mmol) in toluene (300 mL) was heated under reflux overnight at 85 °C. The solvent was removed *in vacuo*, and the residue purified by flash chromatography (SiO_2, 15% Et_2O in pentane, R_f 0.14) to yield a colourless oil **82** (8.52 g, 3.84 mmol, 65%) with identical spectroscopic data to reported values;[47] IR ($CHCl_3$ cast) 2956, 2953, 2931, 1772, 1654, 1076 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.92 (dt, 1H, *J*=15.7, 6.9 Hz, H-2), 5.78 (td, 1H, *J*=15.7, 1.8 Hz, H-3), 4.32 (t, 1H, *J*=5.5 Hz, H-8), 4.15 (q, 2H, *J*=7.0 Hz, H-4), 3.24 (s, 6H, 2 x OCH_3), 2.18 (m, 2H, H-1'), 1.70-1.30 (m, 6H, H-5, H-6, H-7), 1.30 (t, 3H, *J*=6.9 Hz, H-2'); HRMS (ES) calcd for $C_{12}H_{22}O_4$ 230.1518, found 229.1431 $[M-1]^+$.



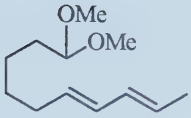
3.2.27 8,8-Dimethoxyoct-2-enol (83).

A modification of the method by Nicolau *et. al.*[40] was employed. 8,8-Dimethoxy-oct-2-enoic acid ethyl ester **82** (5.0 g, 31.25 mmol) was dissolved in CH_2Cl_2 (50 mL) and cooled to –78 °C. Diisobutylaluminum hydride (2.5 M in CH_2Cl_2, 27.50 mL, 68.75 mmol) was added over a 20 min. period, and the resulting mixture stirred for a further 2 h at –78 °C. The reaction was quenched by addition of saturated aqueous NH_4Cl (50 mL), and allowed to warm to room temperature. The reaction mixture was then diluted with Et_2O (500 mL) and filtered through celite. The filtrate was dried ($MgSO_4$) and concentrated *in vacuo* to yield an oil, which was further purified by flash chromatography (SiO_2, 25% EtOAc in hexane, R_f 0.34) to give a colourless oil **83** (3.79 g, 64%) showing identical spectroscopic data to previously reported values;[47] IR ($CHCl_3$ cast) 3417, 2939, 2859, 2832, 1463, 1366 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.20 (m, 2H, H-2, H-3), 4.35 (t, 1H, *J*=5.8 Hz, H-8), 4.10 (m, 2H, H-1), 3.30 (s, 6H, 2 x OCH_3), 2.05 (m, 2H, H-4), 1.65-1.30 (m, 7H, H-5, H-6, H-7, OH); HRMS (ES) calcd for $C_{10}H_{20}O_3$ 188.1412, found 188.1408 $[M]^+$.



3.2.28 8,8-Dimethoxyoct-2-enal (84).

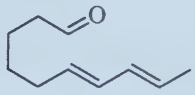
8,8-Dimethoxy-oct-2-enol **83** (2.0 g, 10.62 mmol) was dissolved in CH_2Cl_2 (30 mL) and slowly added to a stirred solution of Dess-Martin reagent **56** (5.16 g, 12.0 mmol) in CH_2Cl_2 (50 mL) at room temperature. After 30 min. of stirring the mixture was diluted with Et_2O (100 mL), and aqueous NaOH (1.3 M, 80 mL) was added. This mixture was stirred for a further 30 min. at room temperature. The organic and aqueous layers were separated and washed with aqueous NaOH (1.3 M, 1 x 50 mL) and H_2O (1 x 50 mL), and then dried over $MgSO_4$. The solvent was removed *in vacuo* to yield an oily residue, which was further purified by flash chromatography (SiO_2, 30% EtOAc in hexane, R_f 0.29) to give a colourless oil **84** (1.92 g, 97%) with identical spectroscopic properties to reported data;[48] ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.46 (d, 1H, *J*=7.9 Hz, H-1), 6.80 (dt, 1H, *J*=15.7, 6.8 Hz, H-3), 6.08 (ddt, 1H, *J*=15.6, 7.9, 1.5 Hz, H-2), 4.32 (t, 1H, *J*=5.7 Hz, H-8), 3.38 (s, 6H, 2 x OCH_3), 2.31 (m, 2H, H-7), 1.62-1.32 (m, 6H, H-4, H-5, H-6); HRMS (ES) calcd for $C_{10}H_{18}O_3$ 186.1256, found 185.1176 $[M-1]^+$.



3.2.29 10,10-Dimethoxydeca-(2,4)-diene (85).

A modification of the method by Schlosser and Christmann[42] was used. Ethyltriphenylphosphonium iodide **61** (4.27 g, 10.20 mmol) was suspended in THF (20 mL) and Et_2O (20 mL). To this suspension was added *n*-butyllithium (2.5 M in hexane, 4.1 mL, 10.20 mmol), and the mixture stirred for 15 min. at room temperature. After that time the mixture turned bright red in colour, and was cooled to –78 °C. 8,8-Dimethoxy-oct-2-enal **84** (1.90 g, 10.20 mmol) in Et_2O (10.0 mL) was slowly added, and stirring continued for an additional 15 min. A second portion of *n*-butyllithium (2.5 M in hexane, 4.1 mL, 10.20 mmol) was added, and stirring continued for a further 5 min. Etheral HCl (1.0 M, 10.20 mL, 10.20 mmol) was added, followed by a quick addition of potassium-*t*-butoxide (1.68 g, 15.0 mmol) in *t*-BuOH (10.0 mL). The mixture was then stirred for 2 h at room temperature, and then it was diluted with Et_2O (400 mL). The etheral layer was then washed with H_2O (4 x 100 mL) and brine (1 x 100 mL), and was dried over $MgSO_4$. Concentration *in vacuo* yielded a yellow oil, which was purified by flash chromatography (SiO_2, 25% EtOAc in hexane, R_f 0.73) to give diene **85** as a colourless oil (1.215 g, 60%); IR ($CHCl_3$ cast) 3016, 2952, 2929, 1457, 1378 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.98 (tdt, 2H, *J*=14.6, 14.6, 0.9 Hz, H-3, H-4), 5.54 (tt, 2H, *J*=6.7, 13.8 Hz, H-2, H-5), 4.33 (t,

1H, *J*=5.7 Hz, H-10), 3.29 (s, 6H, 2 x OCH_3), 2.03 (m, 2H, H-6), 1.70 (d, 3H, *J*=6.3 Hz, H-1), 1.57 (m, 2H, H-9), 1.36 (m, 4H, *J*=6.7 Hz, H-7, H-8); HRMS (ES) calcd for $C_{12}H_{22}O_2$ 198.1620, found 167.1425 $[M\text{-}OCH_3]^+$.



3.2.30 (*E*,*E*)-Deca-6,8-dienal (86).

3.2.30.1 Preparation from acetal (85).

A modification of the method by Roush and Hall[35] was used. 10,10-Dimethoxy-deca-2,4-diene **85** (1.0g, 5.0 mmol) was dissolved in THF (20 mL), and treated with saturated aqueous oxalic acid (15 mL) at room temperature. The mixture was stirred at room temperature for 12, and then a further portion of saturated aqueous oxalic acid (2 mL) was added, and stirring continued for 2 h. The mixture was partitioned between Et_2O (200 mL) and H_2O (50 mL), and the aqueous layer extracted with Et_2O (3 x 50 mL). The Et_2O fractions were combined and washed with 5% aqueous $NaHCO_3$ (1 x 50 mL) and H_2O (1 x 50 mL), and dried ($MgSO_4$) and concentrated *in vacuo* to give a volatile yellow oil which was purified by flash chromatography (SiO_2, 10% Et_2O in hexane, R_f 0.33) to give a colourless oil **86** (0.96g, 4.9 98%); ^1H NMR (300 MHz, $CDCl_3$) δ 9.76 (t, 1H, *J*=1.7 Hz, H-1), 6.00-5.95 (m, 2H, H-7, H-8), 5.64-5.47 (m, 2H, H-6), 2.42 (td, 2H, *J*=7.3, 1.7 Hz), 2.08 (td, 2H, *J*=7.2, 7.2 Hz), 1.72 (d, 3H, *J*=6.4 Hz), 1.64 (tt, 2H, *J*=7.7,

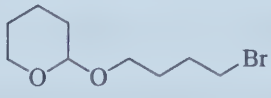
7.3 Hz), 1.42 (tt, 2H, J=7.6, 7.2 Hz); HRMS (ES) calcd for $C_{10}H_{16}O$ 152.1201, found 152.1203 $[M]^+$.

3.2.30.2 Preparation from alcohol (93).

The alcohol **93** (1.34 g, 8.70 mmol) was dissolved in CH_2Cl_2 (30 mL) and slowly added to a stirred solution of Dess-Martin reagent **56** (4.00 g, 9.30 mmol) in CH_2Cl_2 (50 mL) at room temperature. After 30 min. of stirring the mixture was diluted with Et_2O (100 mL), and aqueous NaOH (1.3 M, 80 mL) was added. This mixture was stirred for a further 30 min. at room temperature, and the organic and aqueous layers separated. The organic extract was washed with aqueous NaOH (1.3 M, 1 x 50 mL) and H_2O (1 x 50 mL), and was dried over $MgSO_4$. The solvent was removed *in vacuo* to yield an oily residue, which was further purified by flash chromatography (SiO_2, 10% Et_2O in pentane, R_f 0.39) to give a colourless oil **86** (850 mg, 5.60 mmol, 65%). The spectroscopic data for this oil **86** was identical to that of the aldehyde prepared above.

3.2.31 4-Bromo-1-butanol (88).

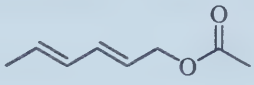
The synthesis of Kang *et. al.*[49] was used. To a solution of 1,4-butanediol (13.68 g, 150 mmol) in benzene (250 mL) was added hydrobromic acid (48%, 18.75 mL). The mixture was heated under reflux for 12 h using a Dean-Starke trap. The mixture was then cooled to room temperature and washed with 6 M NaOH (1 x 150 mL), 10% HCl (1 x 150 mL), H_2O (2 x 200 mL), and brine (1 x 200 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo* to yield a colourless oil **88** (10.40 g, 45%) which could be used without any further purification. ^1H NMR (300 MHz, $CDCl_3$) δ3.66 (t, 2H, J=6.3 Hz), 3.42 (t, 2H, J=6.7 Hz), 1.87 (m, 2H), 1.69 (m, 2H), 1.60 (s, 1H); HRMS (ES) calcd for C_4H_9BrO 151.9837, found 151.9837 $[M]^+$.



3.2.32 2-(4-Bromobutoxy)tetrahydropyran (89).

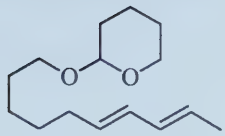
The procedure of Snider and Lu[50] was followed. 3,4-Dihydropyran (4.84 g, 57.5 mmol) and 4-bromo-1-butanol **88** (8.00 g, 52.3 mmol) were dissolved in CH_2Cl_2 (70 mL) and treated with a catalytic amount of *p*-Toluenesulfonic acid (20 mg). The mixture was stirred for 2 hours under argon, and then triethylamine (0.5 mL) was added. The solvent

was removed *in vacuo*, and the residue obtained purified by flash chromatography (SiO_2, 10% EtOAc in hexane, R_f 0.32) to yield a colourless oil **89** (12.91 g, 95%); IR ($CHCl_3$ cast) 2941, 2869, 1727, 1452 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.59 (m, 1H), 3.89-3.73 (m, 2H), 3.54-3.39 (m, 2H), 3.46 (t, 2H, *J*=6.8 Hz), 2.03-2.00 (m, 2H), 1.99-1.70 (m, 4H), 1.68-1.48 (m, 4H); HRMS (ES) calcd for $C_9H_{17}BrO_2$ 236.0412, found 235.0334 $[M-1]^+$.



3.2.33 (*E,E*)-2,4-Hexadienyl acetate (91).

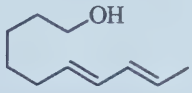
The method of Kulkarni and coworkers[51] was employed. Sorbyl alcohol (5.31 g, 34.4 mmol) was dissolved in THF (100 mL) and cooled to 0 °C. To the above solution was added acetic anhydride (4.21 g, 3.89 mL, 41.3 mmol), followed by triethylamine (4.25 g, 5.85 mL, 42 mmol) and a catalytic amount of pyridine (1.0 mL). This mixture was stirred at 0 °C for 2 h, and then the solvent was removed *in vacuo*. The product was purified by flash chromatography (SiO_2, 10% EtOAc in hexane, R_f 0.41) to yield a colourless oil **91** (3.96 g, 82%); IR ($CHCl_3$ cast) 3024, 2938, 1740, 1662 1103 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.25 (dd, 1H, *J*=15.3, 10.7 Hz), 6.05 (dd, 1H, *J*=15.0, 10.7 Hz), 5.76 (dq, 1H, *J*=15.0, 6.7 Hz), 5.63 (dt, 1H, *J*=15.3, 6.7 Hz), 4.57 (d, 2H, *J*=6.7 Hz), 2.06 (s, 3H), 1.77 (d, 3H, *J*=6.7 Hz); HRMS (ES) calcd for $C_8H_{12}O_2$ 140.0837, found 140.0838 $[M]^+$.



3.2.34 2-Deca-(*E*,*E*)-6,8-dienyloxytetrahydropyran (92).

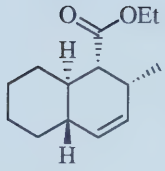
The method of Snider and Lu[50] was followed. To a suspension of magnesium (1.07 g, 44.0 mmol) in dry THF (20 mL) was added dibromoethane (0.5 mL). After completion of the reaction, the solvent was removed by syringe. The magnesium was rinsed with THF (2 x 20 mL), and the THF again removed. A new portion of THF (20 mL) was added, followed by dropwise addition of 2-(4-bromo-butoxy)-tetrahydropyran **89** (6.08g, 25.0 mmol) in THF (20 mL). The mixture was then stirred at room temperature for 2 h. This gray solution was then added dropwise under argon to a cooled (-10 °C) solution of (2*E*,4*E*)-2,4-hexadienyl acetate **91** (2.80 g, 20 mmol) and Li_2CuCl_4 (2.0 M in THF, 20 mL, 20 mmol) in THF (20 mL). The Li_2CuCl_4 was prepared by reacting LiCl (4.24 g, 100 mmol) and $CuCl_2$ (6.72 g, 50 mmol) in THF (250 mL) at room temperature.[52] The colour of the mixture changed from orange to dark brown, to green, and finally to purple. This mixture was stirred under argon at 0 °C for 2 h, and then at room temperature overnight. The reaction was then quenched by addition of saturated aqueous NH_4Cl (50 mL), and extracted with Et_2O (3 x 50 mL). The combined Et_2O fractions were dried ($MgSO_4$) and concentrated *in vacuo* to yield an oil, which was further purified by flash chromatography (SiO_2, 10% EtOAc in hexane, R_f 0.35) to give diene **92** a colourless oil (2.45 g, 51%); ^1H NMR (300 MHz, $CDCl_3$) δ 6.02-5.92 (m, 2H), 5.58-5.46 (m, 2H), 4.59 (m, 1H), 3.90-

3.78 (m, 2H), 3.74-3.64 (m, 2H), 3.52-3.42 (m, 2H), 3.38-3.30 (m, 2H), 2.08-1.98 (m, 2H), 1.70 (d, 3H, *J*=6.4), 1.60-1.44 (m, 4H), 1.40-1.30 (m, 4H); HRMS (ES) calcd for $C_{15}H_{26}O_2$ 238.1933, found 238.1933 $[M]^+$.



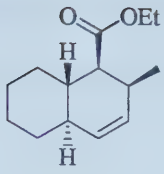
3.2.35 (*E,E*)-Deca-6,8-dienol (93).

2-Deca-(*E,E*)-6,8-dienyloxy-tetrahydro-pyran **92** (2.40 g, 10 mmol) was dissolved in dry MeOH (50 mL) and treated with a catalytic amount of *p*-TsOH (100 mg). The solution was stirred at room temperature for 2 h, and triethylamine (0.5 mL) was added. Most of the solvent was removed *in vacuo*, and the residue taken up in Et_2O (100 mL). The etheral extract was washed with aqueous HCl (1.0 M, 1 x 50 mL) and H_2O (1 x 50 mL), and then dried ($MgSO_4$). Concentration *in vacuo* afforded an oily residue, which was purified by flash chromatography (SiO_2, 20% EtOAc in hexane, R_f 0.19) to yield a colourless oil **93** (1.52 g, 99%); IR ($CHCl_3$ cast) 3416, 3016, 2932, 2855, 1741, 1662 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.00-5.95 (m, 2H), 5.63-5.49 (m, 2H), 4.59 (m, 1H), 3.63 (t, 2H, *J*=6.6 Hz), 2.07 (td, 2H, *J*=6.8, 6.8 Hz), 1.72 (d, 3H, *J*=6.3 Hz), 1.57 (tt, 2H, *J*=7.0, 7.0 Hz), 1.43-1.32 (m, 4H); HRMS (ES) calcd for $C_{10}H_{18}O$ 154.1358, found 154.1352 $[M]^+$.



3.2.36 Ethyl-(1*R*,2*R*,4a*S*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2-methylnaphthalen-1-carboxylate (96) and stereoisomers from oxazolidinone (103).

The oxazolidinone **103** (4.0 mg, 11.3 μmol) was dissolved in CH_2Cl_2 (2.0 mL) and cooled to 0 °C. This solution was then slowly treated with freshly prepared sodium ethoxide (1.0 M in EtOH, 2.0 mL, 2.0 mmol) and stirred for 10 min. at 0 °C. The solution was then allowed to warm to room temperature, and stirred for 10 min. The solvent was removed *in vacuo*, and the residue taken up in $CDCl_3$ (1.0 mL), and filtered through a pasteur pipette containing a cotton wool plug and some silica (5 mm height). The filtrate was subjected to GC-MS analysis to give the following results; 60.3 min (19%, m^+222.16, $C_{14}H_{22}O_2$, **97** or **98**), 60.6 min (15%, m^+222.16, $C_{14}H_{22}O_2$, **97** or **98**), 61.7 min (48%, m^+222.16, $C_{14}H_{22}O_2$, **96**), 61.9 min (18%, m^+222.16, $C_{14}H_{22}O_2$, **95**).



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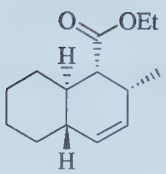
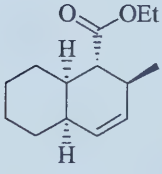


(96)

3.2.37 Racemic mixture of ethyl-(1*S*,2*S*,4a*R*,8a*S*)-1,2,4a,5,6,7,8,8a-octahydro-2-methylnaphthalen-1-carboxylate (95) and ethyl-(1*R*,2*R*,4a*S*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2-methylnaphthalen-1-carboxylate (96).

The method by Roush and Gillis[53] was followed. Dodeca-(*E*,*E*,*E*)-2,8,10-trienoic acid ethyl ester **79** (0.50 g, 2.25 mmol) was dissolved in CH_2Cl_2 (22.0 mL) and slowly over 15 min. treated with ethylaluminium chloride (1.0 M in hexane, 2.14 mL, 2.14 mmol) at room temperature. The mixture was stirred for 3 h at room temperature, and then poured into HCl (1.0 M, 20.0 mL). The aqueous layer was extracted with Et_2O (3 x 30 mL), and the combined Et_2O extracts were dried ($MgSO_4$) and concentrated *in vacuo* to yield a crude mixture of products as a yellow oil. Purification by flash chromatography (SiO_2, 3% Et_2O in pentane, R_f 0.62) afforded a racemic mixture of **95** and **96** a colourless oil (321 mg, 64%). IR ($CHCl_3$ cast) 3012, 2960, 2912, 2877, 2850, 1736 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.50 (ddd, 1H, *J*=10.0, 4.0, 2.6 Hz, H-3), 5.36 (br d, 1H, *J*=9.8 Hz, H-4), 4.11 (q, 2H, *J*=7.1 Hz, H-3'), 2.56-2.47 (m, 2H, H-1, H-2), 1.99-1.87 (m, H-4a), 1.88-1.60 (m, 4H, H-7, H-6), 1.46-1.26 (m, 4H, H-5, H-8), 1.22 (d, 3H, *J*=7.1 Hz, H-4'), 0.89 (d, 3H, *J*=6.9 Hz, 2-CH_3); HRMS (ES) calcd for $C_{14}H_{22}O_2$ 198.1620, found 222.1620

$[M]^+$. The oil was subjected to GC-MS analysis, to give the following results: 61.3 min (50%, m^+222.16, $C_{14}H_{22}O_2$, **95** or **96**), 61.5 min (50%, m^+222.16, $C_{14}H_{22}O_2$, **95** or **96**).



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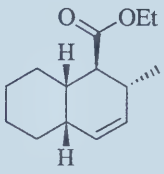
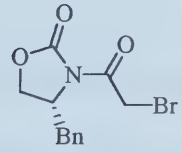


(98)

3.2.38 Racemic mixture of ethyl-(1*R*,2*S*,4a*R*,8a*R*)-1,2,4a,5,6,7,8a-octahydro- 2-methylnaphthalen-1-carboxylate (97) and ethyl-(1*S*,2*R*,4a*S*,8a*S*)-1,2,4a,5,6,7,8a-octahydro- 2-methylnaphthalen-1-carboxylate (98).

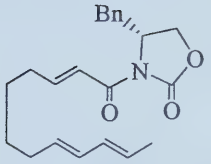
Dodeca-(*E*,*E*,*E*)-2,8,10-trienoic acid ethyl ester **79** (123 mg, 0.55 mmol) was dissolved in toluene (5.0 mL) and heated under reflux for 6 days, until no starting material could be observed by TLC. The solvent was removed *in vacuo*, and the residue purified by preparative TLC (SiO_2, 1% Et_2O in pentane, 3 x elution) to yield two products both as colourless oils (in a 1:1 ratio). The first product was spectroscopically identical to the previously prepared mixture of the two endo cyclized esters **95/96** (38 mg, 31%,), and the second product corresponded to a mixture of the two exo cyclized esters **97/98** (42 mg, 34%), containing ~10% of **95/96**; IR ($CHCl_3$ cast) 3012, 2960, 2912, 2877, 2850, 1736 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.56 (ddd, 1H, *J*=9.9, 4.5, 2.6 Hz, H-3), 5.36 (br d, 1H, *J*=9.9 Hz, H-4), 4.15 (m, 2H, H-3'), 2.58-2.44 (m, 2H, H-1, H-2), 2.10-1.98 (m, H-4), 1.78-1.60 (m, 4H, H-7, H-6), 1.46-1.26 (m, 4H, H-5, H-8), 1.24 (t, 3H, *J*=7.0 Hz, H-4'), 0.94 (d, 3H, *J*=6.9 Hz, 2-CH_3). The oil **97/98** (containing ~10% of **95/96**), was

subjected to GC-MS analysis to give the following results: 59.6 min (40%, m^+222.16, $C_{14}H_{22}O_2$, **97** or **98**), 59.9 (40%, m^+222.16, $C_{14}H_{22}O_2$, **97** or **98**), 61.3 min (10%, m^+222.16, $C_{14}H_{22}O_2$, **95** or **96**), 61.5 (10%, m^+222.16, $C_{14}H_{22}O_2$, **95** or **96**).



3.2.39 (*R*)-4-Benzyl-3-(2-bromo-acetyl)-oxazolidin-2-one (100).

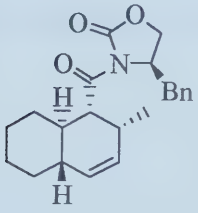
(*R*)-4-Benzyl-2-oxazolidinone (3.54 g, 20 mmol) was dissolved in THF (50 mL) and cooled to –78 °C. This solution was then treated with *n*-BuLi (2.5 M in hexane, 8.0 mL, 20 mmol), and stirred for 30 min. at –78 °C. The above solution was then slowly transferred *via* cannula to a previously prepared solution of bromoacetyl bromide (4.04 g, 2.62 mL, 20 mmol) in THF (20 mL), also at –78 °C. After addition was complete the mixture was stirred for 15 min. at –78 °C, and then the mixture was allowed to warm to room temperature. The solvent was removed *in vacuo*, and the residue was purified by flash chromatography (SiO_2, 50% Et_2O in pentane, R_f 0.37) to yield a colourless oil **100** (5.60 g, 94%); IR ($CHCl_3$ cast) 3028, 2968, 1781, 1700, 1603, 1497, 1478 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ7.29 (m, 5H, Ar-H), 4.72-4.63 (m, 1H, NC<u>H</u>), 4.51 (s, 2H, C<u>H</u>$_2$Br), 4.22 (m, 2H, CHC<u>H</u>$_2$O), 3.30 (dd, 1H, *J*=3.3, 13.5 Hz, 1 x C<u>H</u>-Ar), 2.78 (dd, 1H, *J*=9.6, 13.5 Hz, 1 x C<u>H</u>-Ar); HRMS (ES) calcd for $C_{12}H_{12}BrNO_3$ 297.0001, found 296.9997 $[M]^+$.



3.2.40 (*R*)-4-Benzyl-3-dodeca-(*E*,*E*,*E*)-2,8,10-trienoyl-oxazolidin-2-one (102).

A modification of the method by Evans and Black[54] was used. (*R*)-4-Benzyl-3-(2-bromo-acetyl)-oxazolidin-2-one **100** (1.00 g, 3.35 mmol) was dissolved in THF (6.0 mL) and slowly treated with triphenylphosphine (0.88 g, 3.35 mmol) in THF (2.0 mL). The solution was then stirred overnight at room temperature, and the white solid **101** (1.85 g, 3.30 mmol, 98%) that formed was filtered off and used without any further purification. An amount of this white solid **101** (0.50 g, 0.89 mmol) was dissolved in CH_2Cl_2 (4.0 mL) and cooled to 0 °C. This ice-cold solution was then treated with triethylamine (0.27 g, 0.37 mL, 2.67 mmol) and stirred at 0 °C for 20 min. The solution was allowed to warm to room temperature, and treated with deca-(*E*,*E*)-6,8-dienal **86** (0.125 g, 0.82 mmol) in CH_2Cl_2 (1.0 mL). The mixture was heated under reflux for 3 h, and the solvent removed *in vacuo*. The residue obtained was purified by flash chromatography (SiO_2, 50% Et_2O in pentane, R_f 0.76) to yield an orange oil of compound **102** (193 mg, 67%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.20 (m, 5H, Ar-H), 6.03-5.90 (m, 2H, 2 x C<u>H</u>), 5.66-5.44 (m, 2H, 2 x C<u>H</u>), 4.76-4.64 (m, 1H, NC<u>H</u>), 4.22-4.10 (m, 2H, CHC<u>H</u>$_2$O), 3.32 (dd, 1H, *J*=3.2, 13.4 Hz, 1 x C<u>H</u>-Ar), 2.76 (dd, 1H, *J*=9.5, 13.5 Hz, 1 x C<u>H</u>-Ar), 2.28 (m, 2H, CH_2), 2.05 (m,

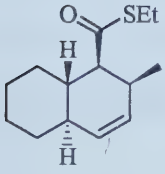
2H, CH_2), 1.70 (d, 3H, J=6.3, CH_3), 1.54-1.36 (m, 4H, 2 x CH_2); HRMS (ES) calcd for $C_{22}H_{27}NO_3$ 353.1991, found 353.1999 $[M]^+$.



3.2.41 (*R*)-4-Benzyl-3- (2-methyl-1,2,4a,5,6,7,8,8a-octahydro-naphthalene-1-carbonyl)-oxazolidin-2-one (103).

The method by Evans and Black[54] was used. (*R*)-4-Benzyl-3-dodeca-(*E*,*E*,*E*)-2,8,10-trienoyl-oxazolidin-2-one **102** (100 mg, 0.28 mmol) was dissolved in CH_2Cl_2 (25.0 mL) and cooled to –78 °C. To this was slowly added dimethylaluminium chloride (1.0 M in hexane, 1.4 mL, 1.4 mmol), and then the solution was allowed to warm to 0 °C. The mixture was stirred for 1 h at 0 °C, and then for 1 h at room temperature. The mixture was re-cooled to 0 °C, and transferred *via* cannula to an aqueous tartaric acid solution (1.0 M, 50.0 mL) at 0 °C. The CH_2Cl_2 fraction was separated, and the aqueous fraction extracted with CH_2Cl_2 (2 x 20 mL). The organic extracts were combined and washed with brine (1 x 50 mL), and then dried over $MgSO_4$. The solvent was removed *in vacuo*, and the residue purified by preparative TLC (SiO_2, 33% Et_2O in pentane, R_f 0.39) to yield a mixture of Diels-Alder adducts as colourless oil (62 mg, 62%), with adduct **103** as the major product; ^{1}H NMR (300 MHz, $CDCl_3$) δ7.38-7.20 (m, 5H, Ar-H), 5.59-5.52 (ddd,

1H, *J*=2.6, 4.5, 9.8 Hz, H-3), 5.38 (br d, 1H, *J*=9.9 Hz, H-4), 4.74-4.65 (m, 1H, NC<u>H</u>), 4.12 (m, 2H, CHC<u>H</u>$_2$O), 3.80 (dd, 1H, *J*=11.3, 5.9 Hz, 1xC<u>H</u>-Ar), 3.40 (dd, 1H, *J*=3.3, 13.1 Hz, 1xC<u>H</u>-Ar), 2.72-2.84 (m, 1H, CH), 2.61 (dd, 1H, *J*=10.5, 13.1, CH), 1.88 (d, 1H, *J*=9.8, CH), 1.74 (m, 4H, H-7, H-6),1.64-1.00 (m, 8H,4 x CH_2), 0.93 (d, 3H, J=7.2 Hz, 2-CH_3); HRMS (ES) calcd for $C_{22}H_{27}NO_3$ 353.1991, found 353.1993 $[M]^+$.



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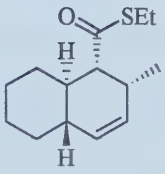
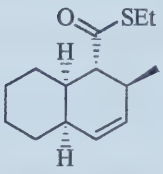


(105)



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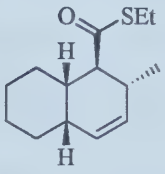


(107)

3.2.42 Thioethyl-(1*S*,2*S*,4a*R*,8a*S*)-1,2,4a,5,6,7,8,8a-octahydro-2-methylnaphthalen-1-carboxylate (104), thioethyl-(1*R*,2*R*,4a*S*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2-methylnaphthalen-1-carboxylate (105), thioethyl-(1*R*,2*S*,4a*R*,8a*R*)-1,2,4a,5,6,7,8a-octahydro- 2-methylnaphthalen-1-carboxylate (106) and thioethyl-(1*S*,2*R*,4a*S*,8a*S*)-1,2,4a,5,6,7,8a-octahydro- 2-methylnaphthalen-1-carboxylate (107).

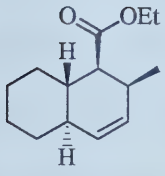
Dodeca-(*E*,*E*,*E*)-2,8,10-trienethoic acid *S*-ethyl ester **80** (133 mg, 0.50 mmol) was dissolved in toluene (5.0 mL) and heated under reflux for 6 days, until no starting material could be observed by TLC. The solvent was removed *in vacuo*, and the residue purified by flash chromatography (SiO_2, 0.5% Et_2O in petroleum ether) to yield two products (in a 1:1 ratio) as colourless oils. The first product was a racemic mixture of the

two *endo* cyclized esters **104/105** (5.3 mg, 31%); IR ($CHCl_3$ cast) 3012, 2960, 2912, 2877, 2850, 1736 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.55-5.48 (ddd, 1H, *J*=9.9, 4.5, 2.5 Hz, H-3), 5.35 (br d, 1H, *J*=10.0 Hz, H-4), 2.84 (q, 2H, H-3'), 2.55 (m, 1H, H-1), 1.84 (m, 1H, H-4), 1.70 (m, 4H, H-7, H-6), 1.46-1.26 (m, 4H, H-5, H-8), 1.24 (t, 3H, *J*=7.0 Hz, H-4'), 0.83 (d, 3H, *J*=7.1 Hz, 2-CH_3), HRMS (ES) calcd for $C_{14}H_{22}OS$ 238.1391, found 222.1620 $[M]^+$.

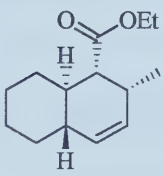
The second product corresponded to a racemic mixture of the two *exo* cyclized esters **106/107** (42 mg, 34%); IR ($CHCl_3$ cast) 3012, 2960, 2912, 2877, 2850, 1736 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.62-5.54 (ddd, 1H, *J*=9.8, 4.9, 1.9 Hz, H-3), 5.35 (dt, 1H, *J*=9.9, 1.5 Hz, H-4), 2.90 (q, 2H, *J*=7.4 Hz, H-3'), 2.54 (m, 2H, H-1, H-2), 2.14 (m, 1H, 1 x H-4), 2.00 (m, 1H, 1 x H-4), 1.66-1.40 (m, 4H, H-7, H-6), 1.24 (t, 3H, *J*=7.4 Hz, H-4'), 0.97 (d, 3H, *J*=6.7 Hz, 2-CH_3); HRMS (ES) calcd for $C_{14}H_{22}OS$ 238.1391, found 222.1620 $[M]^+$. The respective mixtures of oils (**104**, **105**, **106**, **107**), (**104**, **105**), (**106**, **107**), were subjected to GC-MS analysis. No separation of any of the thioesters could be achieved.

3.3 Enzymatic studies

3.3.1 GC-MS method development.



(95)



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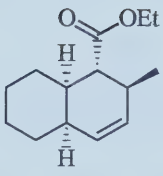
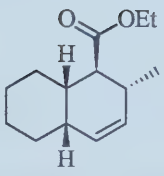


(97)



(98)

3.3.1.1 Column conditions and method used in GC-MS analysis.

A flow of 1.0 mL/min. with an injection split of 10:1 and temperature programming starting at 80 °C, increasing by 1 °C/min. to 160 °C, and then by 10 °C/min. to 200 °C. The column temperature was maintained at 200 °C for 36 min., to complete the run. This method gave good separation of a 1:1:1:1 ratio of a mixture of the 4 above compounds, with elution times of 59.6 min. (25%, $[M^+]$ 222.16, $C_{14}H_{22}O_2$, **97** or **98**), 59.9 min. (25%, $[M]^+$ 222.16, $C_{14}H_{22}O_2$, **97** or **98**). 61.3 min. (25%, $[M]^+$ 222.16, $C_{14}H_{22}O_2$, **95** or **96**), 61.4 min. (25%, $[M]^+$ 222.16, $C_{14}H_{22}O_2$, **95** or **96**). The identity of the expected enzymatic product (**95**) was eluded by injection of a mixture of **95/96** to give retention times of 61.3 min (50%, $[M]^+$ 222.16, $C_{14}H_{22}O_2$, **95** or **96**), 61.4 min. (50%, $[M]^+$ 222.16, $C_{14}H_{22}O_2$, **95** or **96**). Injection of only the synthesized enantiomer (**96**), containing (~15-20% each of **95, 97** and **98**), gave retention times of 60.4 min. (19%, $[M]^+$ 222.16,

$C_{14}H_{22}O_2$, **97** or **98**), 60.5 min. (15%, $[M]^+$ 222.16, $C_{14}H_{22}O_2$, **97** or **98**), 61.6 min. (48%, $[M]^+$ 222.16, $C_{14}H_{22}O_2$, **96**), 61.9 min. (18%, $[M]^+$ 222.16, $C_{14}H_{22}O_2$, **95**).

3.3.2 Fermentation of the *Aspergillus nidulans lovB* strain.

Aspergillus nidulans LovB strain was provided by Prof. C.R. Hutchinson (University of Wisconsin).[21] The *A. nidulans LovB* strain (UAMH 8965 or WMH738) had been transformed with a plasmid containing the uridine marker, as well as the lovastatin nonaketide synthase (LNKS) gene of the lovastatin gene cluster under control of the *alcA* promotor. PABA stock solution (needed for growth) was prepared by dissolving *p*-aminobenzoic acid (0.1 g) in Milli-Q H_2O (100 mL). The 10 x AMM salt solution was prepared by dissolving $NaNO_3$ (60.0 g), KCl (5.2 g) and KH_2PO_4 (15.2 g) in Milli-Q H_2O (diluted to 1000 mL), and adjusting to pH 6.5. The trace elements solution (required for the production medium) contained $FeSO_4{\cdot}7H_2O$ (1.0 g), $ZnSO_4{\cdot}7H_2O$ (8.8 g), $CuSO_4{\cdot}5H_2O$ (0.4 g), $MnSO_4{\cdot}4H_2O$ (0.15 g), $Na_2B_4O_7{\cdot}10H_2O$ (0.1 g), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.05 g), and concentrated HCl (0.5 mL), diluted to 1000 mL with Milli-Q water. Agar complete medium contained malt extract (20.0 g), bacto-peptone (1.0 g), glucose (20.0 g), agar (20.0 g) and PABA stock solution (1.0 mL), diluted to 1.0 L with Milli-Q water. The ACM slants were inoculated with the the *A. nidulans LovB* strain, and incubated for 5 days at 30 °C, and then stored at 4 °C. The yeast extract production medium (YEPD) contained yeast extract (20.0 g), bacto-peptone (1.0 g), glucose (20.0 g) and PABA stock solution (1.0 mL), diluted to 1.0 L with Milli-Q water. This medium was inoculated with one of the prepared slants, and fermented (1.0 L in a

2.0 L Erlenmeyer flask) at 180 rpm and 30 °C for 3 days. Myracloth (Calbiochem, La Jolla, CA) was used to filter the mycelia, which was then washed with AMM production medium (2.0 L), and transferred to fresh AMM production medium (1.0 L in a 2.0 L Erlenmeyer flask). This was then incubated at 180 rpm and 30 °C for 3 days. The AMM production medium used, consisted of trace elements solution (1.0 mL), 10 x AMM salt solution (100.0 mL), PABA stock solution (1.0 mL), and cyclopentanone for induction (0.9 mL) diluted to 1.0 L with Milli-Q water. This was autoclaved, and then treated with sterile 25% $MgSO_4 \cdot 7H_2O$ (2.5 mL) and sterile 40% lactose solution (25.0 mL).

3.3.3 Isolation and purification of *lovB* protein.

The method of Reeves[1a] was used. The mature culture (1.0 L) of the *A. nidulans LovB* strain mentioned above, was gravity filtered through Miracloth, and the mycelia washed with Milli-Q water (4.0 L) and squeezed as dry as possible. These wet mycelia (ca. 15.0 g) were frozen (–78 °C) and the freeze-dried overnight. All the following steps were done at 4 °C. The dried mycelia (ca. 3.0 g) were pulverized (using a mortar and pestle for a minimum of 15 min.) in extraction buffer (25 mL), consisting of Tris-HCl (3.15 g, 20 mM) pH 8.0 (using 1.0 M NaOH), glycerol (100.0 mL, 10%), NaCl (58.44 g, 1.0 M), sodium ascorbate (0.99 g, 5 mM), EDTA (0.372 g, 1.0 mM), DTT (0.154 g, 1.0 mM), leupeptin (0.0038 g, 3.8 μg/mL), chymostatin (0.0177 g, 17.7 μg/mL), pepstatin (0.0020 g, 2.0 μg/mL), trypsin inhibitor (0.042 g, 42.0 μg/mL), phenylmethylsulfonyl fluoride (0.035 g, 0.2 mM), and polyvinylpolypyrrolidone (22.0 g, 2.2%), all diluted to 1.0 L with Milli-Q water. The broken cell suspension was diluted with more extraction buffer (175.0

mL) and left on a orbital shaker for 1 hour at 75 rpm. The cell debris was separated by centrifuging at 10000 x g for 30 min. and then the supernatant was gravity filtered through 4 layers of cheesecloth, to afford the cell free extract (CFE). This CFE (150.0 mL) was subjected to dialysis using an Amicon 200 apparatus with a YM-100 membrane (100 000 MW cutoff). The CFE was concentrated to approximately 20 mL and was then loaded onto a Sephadex G-150 column (1.0 L), equilibrated at 4 °C with the elution buffer consisting of Tris-HCl (6.304 g, 20 mM), glycerol (200 mL, 10%), NaCl (11.69 g, 0.1 M), sodium ascorbate (0.396 g, 1.0 mM), EDTA (0.745 g, 1.0 mM), DTT (0.308 g, 1.0 mM), and Tween80 (2.0 mL, 0.1%), diluted to 2.0 L with Milli-Q water. Fractions of 8.0 mL were collected at a flow of 0.8 mL/min. The protein concentration was monitored by UV at 280 nm. Fractions of protein were combined and precipitated by adding ammonium sulfate to 60% saturation. The precipitated protein was isolated by centrifuging twice at 9000 x g for 30 min., and removal of the supernatant by Pasteur pipette. The collected protein was resuspended in a minimum amount of pellet buffer (ca. 10.0 mL), consisting of Tris-base (0.606 g, 20 mM) pH 8.0 (using 1.0 M NaOH), DTT (0.038 g, 1.0 mM), and sodium ascorbate (0.050 g, 1 mM) diluted to 250.0 mL with Milli-Q water. Aliquots of this protein in pellet buffer was used for SDS-PAGE gels, and for the enzyme assay of the ~335 kDa protein.

3.3.4 SDS-PAGE gels of *lovB* protein.

The Bio-Rad 5% SDS-PAGE Ready Gels were ran at 150 V for 80 min. with a running buffer solution consisting of Tris-base (15.0 g), glycine (72.0 g) and sodium dodecyl

sulfate (5.0 g), diluted to 1.0 L with Milli-Q water. Of this 1.0 L stock solution 100.0 mL aliquots were taken and diluted to 500.0 mL to obtain fresh running buffer. The gels were visualized by staining using Coomassie Blue stain solution supplied by Biorad. Stained gels were destained with a solution prepared by adding acetic acid (70.0 mL) to EtOH (300.0 mL), and diluting to 1.0 L with Milli-Q water.

3.3.5 Activity assay for the *lovB* protein.

A cofactor solution was prepared, containing SAM (1.0 mg), NADPH (1.0 mg) and FAD (1.0 mg) in Milli-Q water (1.0 mL). A blank control was prepared by adding together the cofactor solution (20.0 μL), fresh enzyme solution in pellet buffer (500.0 μL), and pellet buffer (480.0 μL). The test solution was prepared by adding the cofactor solution (20.0 μL) to fresh enzyme solution in pellet buffer (500.0 μL), pellet buffer (180.0 μL), acetyl CoA solution (50.0 μL, 1.0 mg/mL solution) and malonyl CoA solution (250.0 μL, 5.0 mg/mL solution). The activity of the enzyme was established by monitoring pigment formation[19] at 365 nm over 16 h.

3.3.6 Testing of compounds with purified *lovB* enzyme.

3.3.6.1 Testing of (6*R*)-(*E,E,E*)-6-methyldodeca-2,8,10-trienoic acid *N*-acetylcysteamine thioester (40) with *lovB* enzyme.

(6*R*)-(*E,E,E*)-6-Methyldodeca-2,8,10-trienoic acid *N*-acetylcysteamine thioester **40** (5.0 mg, 0.016 mmol) was dissolved in MeOH (180.0 μL) and slowly added to fresh enzyme solution in pellet buffer (2.0 mL). This was then gently swirled on an orbital shaker at room temperature for 3 days. A further portion of enzyme solution (1.0 mL) was added, and swirling continued for an additional day. The reaction mixture was then diluted with H_2O (5.0 mL) and extracted with EtOAc (2 x 5.0 mL), followed by extraction with $CHCl_3$ (2 x 5.0 mL). The combined organic fractions were then dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (0.5 mL) and cooled to 0 °C. This residue was then treated with a sodium ethoxide/ethanol solution (0.5 M, 50 μL, 0.025 mmol). The reaction mixture was allowed to warm to room temperature, and was then heated to 60 °C overnight. After cooling to room temperature the reaction was quenched by addition of H_2O (10.0 mL). This was then extracted with $CHCl_3$ (6 x 5.0 mL), and all organic fractions combined, dried ($MgSO_4$) and concentrated *in vacuo*. Purification by preparative TLC (SiO_2, 20% EtOAc in hexane) yielded an ethyl ester which showed identical spectroscopic properties as the reference ethyl ester **43** (0.1

mg, 2% over two steps), as well as a mixture of the ethyl esters **45** and **47** (3.2 mg, 60% over 2 steps).

3.3.6.2 Testing of (6*R*)-(*E,E,E*)-6-methyldodeca-2,8,10-trienoic acid *N*-acetylcysteamine thioester (40) with denatured *lovB* enzyme.

A solution of the LNKS enzyme (3.0 mL, in pellet buffer) was boiled at 100 °C for 1 h. Analysis using the UV assay described above, showed the enzyme to be inactive. (6*R*)-(*E,E,E*)-6-Methyldodeca-2,8,10-trienoic acid *N*-acetylcysteamine thioester **40** (4.0 mg, 0.013 mmol) was dissolved in MeOH (150.0 μL) and slowly added to this denatured enzyme solution (2.0 mL). This was then gently swirled on an orbital shaker at room temperature for 3 days. A further portion of denatured enzyme solution (1.0 mL) was added, and swirling continued for an additional day. The reaction mixture was then diluted with H_2O (5.0 mL) and extracted with EtOAc (2 x 5.0 mL), followed by extraction with $CHCl_3$ (2 x 5.0 mL). The combined organic fractions were then dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (0.5 mL) and cooled to 0 °C. This residue was then treated with a sodium ethoxide/ethanol solution (0.5 M, 50 μL, 0.025 mmol). The reaction mixture was allowed to warm to room temperature, and was then heated to 60 °C overnight. After cooling to room temperature the reaction was quenched by addition of H_2O (10.0 mL). This was then extracted with $CHCl_3$ (6 x 5.0 mL), and all organic fractions combined, dried ($MgSO_4$) and concentrated *in vacuo*. Purification by preparative TLC (SiO_2, 20% EtOAc in hexane) yielded a mixture of the ethyl esters **45** and **47** (1.9 mg, 62% over 2 steps).

3.3.6.3 Testing of ethyl-(6*R*)-(*E,E,E*)-6-methyldodeca-2,8,10-trienoate (41) with *lovB* enzyme.

Ethyl (6*R*)-(*E,E,E*)-6-Methyldodeca-2,8,10-trienoate **41** (5.0 mg, 0.016 mmol) was dissolved in MeOH (180.0 μL) and slowly added to fresh enzyme solution in pellet buffer (2.0 mL). This was then gently swirled on an orbital shaker at room temperature for 3 days. A further portion of enzyme solution (1.0 mL) was added, and swirling continued for 1 more day. The reaction mixture was then diluted with H_2O (5.0 mL) and extracted with EtOAc (2 x 5.0 mL), followed by extraction with $CHCl_3$ (2 x 5.0 mL). The combined organic fractions were then dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (0.5 mL) and cooled to 0 °C. This residue was then treated with a sodium ethoxide/ethanol solution (0.5 M, 50 μL, 0.025 mmol). The reaction mixture was allowed to warm to room temperature, and was then heated to 60 °C overnight. After cooling to room temperature the reaction was quenched by addition of H_2O (10.0 mL). This was then extracted with $CHCl_3$ (6 x 5.0 mL), and all organic fractions combined, dried ($MgSO_4$) and concentrated *in vacuo*. Purification by preparative TLC (SiO_2, 3% Et2O in hexane, R_f0.25) yielded a mixture of only ethyl esters **45** and **47** (4.1 mg, 82%).

3.3.6.4 Testing of (6*R*)-methyldodeca-(*E*,*E*,*E*)-2,8,10-trienethioic acid *S* ethyl ester (77) with *lovB* enzyme.

6-(*R*)-Methyl-dodeca-(*E*,*E*,*E*)-2,8,10-trienethioic acid *S*-ethyl ester **77** (5.0 mg, 0.016 mmol) was dissolved in MeOH (180.0 μL) and slowly added to fresh enzyme solution in pellet buffer (2.0 mL). This was then gently swirled on an orbital shaker at room temperature for 3 days. A further portion of enzyme solution (1.0 mL) was added, and swirling continued for an additional day. The reaction mixture was then diluted with H_2O (5.0 mL) and extracted with EtOAc (2 x 5.0 mL), followed by extraction with EtOAc (2 x 5.0 mL) and with $CHCl_3$ (2 x 5.0 mL). The combined organic fractions were then dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (0.5 mL) and cooled to 0 °C. This residue was then treated with a sodium ethoxide/ethanol solution (0.5 M, 50 μL, 0.025 mmol). The reaction mixture was allowed to warm to room temperature, and was then heated to 60 °C overnight. After cooling to room temperature the reaction was quenched by addition of H_2O (10.0 mL). This was then extracted with $CHCl_3$ (6x5.0 mL), and all organic fractions combined, dried ($MgSO_4$) and concentrated *in vacuo*. Purification by preparative TLC (SiO_2, 3% Et_2O in hexane, R_f0.25) yielded a mixture of only ethyl esters **45** and **47** (4.0 mg, 80%).

3.3.6.5 Testing of dodeca-(*E*,*E*,*E*)-2,8,10-trienethoic acid *S*-(2-acetylamino-ethyl) ester (78) with *lovB* enzyme.

Varying concentrations of dodeca-(*E*,*E*,*E*)-2,8,10-trienethoic acid *S*-(2-acetylamino-ethyl) ester **78** was tested with the *LovB* enzyme. The following amounts of compound

were tested: (6.0 mg, 0.016 mmol), (3.0 mg, 0.008 mmol), (1.5 mg, 0.004 mmol), and (0.75 mg, 0.0002 mmol). Each amount of material was dissolved in MeOH (150.0 μL) and slowly added to fresh enzyme solution in pellet buffer (2.0 mL). This was then gently swirled on an orbital shaker at room temperature for 3 days. A further portion of enzyme solution (1.0 mL) was added to each vial, and swirling continued for 1 more day. The respective reaction mixtures were then diluted with H_2O (5.0 mL) and extracted with EtOAc (2 x 5.0 mL), followed by extraction with $CHCl_3$ (2 x 5.0 mL). The combined organic fractions for each test sample were then dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residues were dissolved in CH_2Cl_2 (0.5 mL) and cooled to 0 °C. These residues were then treated with a sodium ethoxide/ethanol solution (0.5 M, 50 μL, 0.025 mmol). The reaction mixtures were allowed to warm to room temperature, and were then heated to 60 °C overnight. After cooling to room temperature the reactions were quenched by addition of H_2O (10.0 mL). This was then extracted with $CHCl_3$ (6x5.0 mL), and all organic fractions combined, dried ($MgSO_4$) and concentrated *in vacuo*. The residues were dissolved in $CDCl_3$ (250 μL) and subjected to the GC-MS analysis. For all concentrations of material tested 4 compounds corresponding to the ethyl esters **95**, **96**, and **97, 98** were observed, eluting at retention times of 59.6 min. ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **97** or **98**), 59.9 min. ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **97** or **98**), 61.2 min. ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **96**), 61.4 min. ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **95**). The compounds were present in a 1:1:1:1 ratio, with no significant enhancement of the compound eluting at 61.4min. (**95**).

3.3.6.6 Testing of dodeca-(*E,E,E*)-2,8,10-trienoic acid ethyl ester (79) with *lovB* enzyme.

Dodeca-(*E,E,E*)-2,8,10-trienoic acid ethyl ester **79** (5.0 mg, 0.016 mmol) was dissolved in MeOH (180.0 μL) and slowly added to fresh enzyme solution in pellet buffer (2.0 mL). This was then gently swirled on an orbital shaker at room temperature for 3 days. A further portion of enzyme solution (1.0 mL) was added, and swirling continued for an additional day. The reaction mixture was then diluted with H_2O (5.0 mL) and extracted with EtOAc (2 x 5.0 mL), followed by extraction with $CHCl_3$ (2 x 5.0 mL). The combined organic fractions were then dried ($MgSO_4$) and concentrated *in vacuo*. The residue was dissolved in $CDCl_3$ (250 μL) and subjected to the GC-MS analysis. Four compounds, corresponding to the ethyl esters **95**, **96**, and **97, 98** were observed eluting at retention times of 59.6 min ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **97** or **98**), 59.9 min. ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **97** or **98**), 61.2 min ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **96**), 61.4 min. ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **95**). The compounds were present in a 1:1:1:1 ratio, with no significant enhancement of the compound eluting at 61.4 min. (**95**).

3.3.6.7 Testing of dodeca-(*E,E,E*)-2,8,10-trienethoic acid *S*-ethyl ester (80) with *lovB* enzyme

Dodeca-(*E,E,E*)-2,8,10-trienoic acid *S*-ethyl ester **80** (5.0 mg, 0.016 mmol) was dissolved in MeOH (180.0 μL) and slowly added to fresh enzyme solution in pellet buffer (2.0 mL). This was then gently swirled on an orbital shaker at room temperature for 3 days. A further portion of enzyme solution (1.0 mL) was added, and swirling continued for an

additional day. The reaction mixture was then diluted with H_2O (5.0 mL) and extracted with EtOAc (2 x 5.0 mL), followed by extraction with $CHCl_3$ (2 x 5.0 mL). The combined organic fractions were then dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (0.5 mL) and cooled to 0 °C. This was then treated with a sodium ethoxide/ethanol solution (0.5 M, 50 μL, 0.025 mmol). The reaction mixture was allowed to warm to room temperature, and was then heated to 60 °C overnight. After cooling to room temperature the reaction was quenched by addition of H_2O (10.0 mL). This was then extracted with $CHCl_3$ (6x5.0 mL), and all organic fractions combined, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was dissolved in $CDCl_3$ (250μL) and subjected to the GC analysis, as discussed above. Four compounds corresponding to the ethyl esters **95**, **96**, and **97, 98** were observed eluting at retention times of 59.6 min. ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **97** or **98**), 59.9 min. ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **97** or **98**), 61.2 min. ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **96**), 61.4 min. ($[M]^+$ 222.16, $C_{14}H_{22}O_2$, **95**). The compounds were present in a 1:1:1:1 ratio, with no significant enhancement of the compound eluting at 61.4 min. (**95**).

4 REFERENCES

[1] (a) Hendrickson, L., Davis, C.R., Roach, C., Nguyen, D.K., Aldrich, T., McAda, P.C., Reeves, C.D. *Chem. Biol.* **1999**, *6*, 429-439. (b) Moore, R.N., Bigham, G., Chan, J.K., Hogg, A.M., Nakashima, T.T., Vederas, J.C. *J. Am. Chem. Soc.* **1985**, *107*, 3694-3701. (c) Greenspan, M.D., Yudkovitz, J.B., *J. Bacteriol.* **1985**, *162*, 704-707. (d) Endo, A., Negishi, Y., Iwashita, T., Mizukawa, K., Hirama, M. *J. Antibiot.* **1985**, *38*, 444-448. (e) Shiao, M.S., Don, H.,S. *Proc. Natl. Sci. Counc. Repub. China, Part B: Life Sci.* **1987**, *11*, 223-231. (f) Yoshizawa, Y., Witter, D.J., Liu, Y., Vederas, J.C. *J. Am. Chem. Soc.* **1994**, *116*, 2693-2694. (g) Wagschal, K., Yoshizawa, Y., Witter, D.J., Liu, Y., Vederas, J.C. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2357-2363. (h) Witter, D.J., Vederas, J.C. *J. Org. Chem.* **1996**, *61*, 2613-2623.

[2] Alberts, A.W., Chen, J., Kuron, G., Hunt, V., Huff, J., Hoffman, C., Rothrock, J., Lopez, M., Joshua, H., Harris, E., Patchett, A., Monaghan, R., Currie, S., Stapely, E., Alberts-Schonberg, G., Hensens, O., Hirshfield, J., Hoogsteen, K., Liesch, J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3957-3961.

[3] (a) Endo, A. Hasumi, K., *J. Antibiot.* **1979**, *32*, 852-854. (b) Brown, A.G., Smale, T.C. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1165-1170.

[4](a) Staunton, J., Wilkinson, B. In *Biosynthesis Polyketides and Vitamins; The Biosynthesis of Aliphatic Polyketides; Topics in Current Chemistry*, Vol. 195; Leeper, F.J., Vederas, J.C., Eds.; Springer Verlag Berlin Heidelberg: Cambridge, **1998**, 49-92. (b) Cane, D.E., Walsh, C.T., Khosla, C. *Science* **1998**, *282*, 63-68. (c) Hopwood, D.A.,

Chem. Rev. **1997**, *97*, 2465-2497. (d) Hutchison, C.R., Fujii, I., *Annu. Rev. Microbiol.* **1995**, *49*, 201-238. (e) Rohr, J., *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 881-885. (f) Katz, L., Donadio, S., *Annu. Rev. Microbiol.* **1993**, *47*, 875-912. (g) Hopwood, D.A., Sherman, D.H., *Annu. Rev. Genet.* **1990**, *24*, 37-66. (h) Eckermann, S., Schröder, G., Schmidt, J., Strack, D., Edrada, R.A., Helariutta, Y., Elomaa, P., Kotilainen, M., Kipeläinen, Proksch, P., Teeri, T.H., Schröder, J. *Nature* **1998**, *396*, 387-390. (i) Schröder, J., *Nature Struct. Biol.* **1999**, *6*, 714-716. (j) Ferrer, J.L., Jez, J.M., Bowman, M.E., Dixon, R.A., Noel, J.P. *Nature Struct. Biol.* **1999**, *6*, 775-784. (k) Rohr, J. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 2847-2849.

[5] Grundy, S.M., Vega, G.L., *J. Lipids Res.* **1985**, *26*, 1464-1475.

[6] Stryer, L., In *Biochemistry*, W.H. Freeman and Company: New York, **1988**, 554-564.

[7] Bell, F.P., In *Pharmacological Control of Hyperlipidaemia*. Fears, R. (Eds.), Prous Science Publishers, Barcelona, Spain **1986**, 409.

[8] Beale, M.H. *Nat. Prod. Rep.* **1991**, *8*, 441-454.

[9] Endo, A., Kuroda, M. Tsujita, Y., *J. Antibiot.* **1976**, *29*, 1346-1348.

[10] Brown, A.G., Smale, T.C., King, T.J., Hasenkamp, R., Thompson, R.H. *J.Chem. Soc. Perkin Trans. 1* **1976**, 1165-1170.

[11] Endo, A., *J. Med. Chem.* **1985**, *28*, 401-405.

[12] (a) Witzum, J.L., In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, (9th ed., Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., Gilman, A.G., Eds., McGraw-Hill: New York, **1996**, 885-887. (b) Endo, A., Hasumi, K.

Nat. Prod. Rep. **1993**, *10*, 541-550. (c) Christians, U., Jacobsen, W., Floren, L.C. *Pharmcol. Ther.* **1998**, *80*, 1-34.

[13] Hoffman, W.F., Alberts, A.W., Anderson, P.S., Chen, J.S., Smith, R.L., Willard, A.K., *J. Med. Chem.* **1986**, *29*, 849-852.

[14] Tsujita, Y., Kuroda, M., Shimada, Y., Tanzawa, K., Arai, M., Kaneko, I., Tanaka, M., Masuda, H., Tarumi, C., Watanabe, Y., Fujii, S. *Biochim. Biophys. Acta* **1986**, *877*, 50-60.

[15] Yuan, J.N., Tsai, M.Y., Hegland, J., Hunninghake, D.B. *Atherosclerosis* **1991**, *87*, 147-157.

[16] Angerbauer, R., Fey, P., Hubsch, W., Phillips, T., Schmidt, D. *XI International Symposium on Drugs Affecting Lipid Metabolism*, Florence, Italy **1992**,69.

[17] Roth, D.B., Blankley, C.J., Chucholowski, A.W., Ferguson, E., Hoefle, M.L., Ortwine, D.F., Newton, R.S., Sekerke, C.S., Sliskovic, D.R., Stratton, C.D., Wilson, M.W. *J. Med. Chem.* **1991**, *34*, 357-366.

[18] Newman, D.J., Laird, S.A. In *The Commercial Use of Biodiversity.*, Laird, S.A. Ed., Earthscan Publications, London, U.K. **1999**, 333.

[19] Auclair, K., Ph.D. thesis **1999**.

[20] Chan, J.K., Moore, R.N., Nakashima, T.T., Vederas, J.C. *J. Am. Chem Soc.* **1983**, *105*, 3334-3336.

[21] Kennedy, J., Auclair, K., Kendrew, S.G., Park, C., Vederas, J.C., Hutchinson, C.R. *Science* **1999**, *284*, 1368-1372.

[22] Oikawa, H., Suzuki, Y., Katayama, K., Nya, A., Sakano, C., Ichihara, A. *J. Chem. Soc. Perkin Trans. 1*, **1999**, 1225-1232.

[23] Ichihara, A., Oikawa, H., In *Comprehensive Natural Products Chemistry. Polyketides and Other Secondary Metabolites.*, Sankawa, U., Barton, D., Nakanishi, K., Meth-Cohn, O. (series Eds.), Elsevier, New York, N.Y., U.S.A. **1999**, 1:367-408.

[24] Heine, A., Stura, E.A., Yli-Kauhhaluoma, J.T., Gao, C., Deng, Q., Beno, B.R., Houk, K.N., Janda, K.D., Wilson, I.A. *Science* **1998**, *279*, 1934-1940.

[25] Tarasow, T.M., Tarasow, S.L., Eaton, B.E. *J. Am. Chem. Soc.* **2000**, *122*, 1015-1021.

[26] Oikawa, H., Kobayashi, T., Katayama, K., Suzuki, Y., Ichihara, A. *J. Org. Chem.* **1998**, *63*, 8748-8756.

[27] Dess, B.D., Martin, J.C. *J. Org. Chem.* **1983**, *48*, 4155-4156.

[28] (a) Frigerio, M., Santagostino, M., Sputore, S. *J. Org. Chem.* **1999**, *64*, 4537-4538. (b) Ireland, R.E., Liu, L.J. *J. Org. Chem.* **1993**, *58*, 2899. (c) Meyer, S.D., Schreiber, S.L. *J. Org. Chem.* **1994**, *59*, 7549.

[29] Witter, D.J. Ph.D. thesis **1994**.

[30] Ager, D.J., Prakash, I., Schaad, D.R. Aldrichimica Acta **1997**, *30*, 3-12.

[31] Auclair, K., Sutherland, A., Kennedy, J., Witter, D.J., van den Heever, J.P., Hutchinson, C.R., Vederas, J.C. *J. Am. Chem. Soc.* **2000,** *122*, 11519-11520.

[32] Perrin, D.D., Armarego, W.L.F., Perrin, D.R., *Purification of Laboratory Chemicals*, 2nd Ed., Pergamon: New York, **1980**.

[33] Still, W.C., Kahn, M., Mitra, A.J., *J. Org. Chem.* **1978**, *43*, 2923-2925.

[34] Parker, D., *J. Chem. Soc., Perkin Trans. II* **1983**, 83-88.

[35] Roush, W.R., Hall, S.E., *J. Am. Chem. Soc.* **1981**, *103*, 5200-5211.

[36] Stork, G., Nakahara, Y., Greenlee, W.J., *J. Am. Chem. Soc.* **1978**, *100*, 7775-77777.

[37] Claus, R.E., Schreiber, S.L., *Org. Synth. Coll. Vol. VII*, **1990**, 168-171.

[38] Isler, O., Gutmann, H., Montavon, M., Rüegg, R., Ryser, G., Zeller, P., *Helv. Chim. Acta*, **1957**, *40*, 1242-1249.

[39] Hungerbühler, E., Seebach, D., Wasmuth, D., *Helv. Chim. Acta* **1981**, *64*, 1467-1487.

[40] Nicolaou, K.C., Daines, R.A., Uenishi, J., Li, W.S., Papahatjis, D.P., Chakraborty, T.K., *J. Am. Chem. Soc.* **1988**, *110*, 4672-4685.

[41] Barnhardt, R.G. Jr, McEwan, W.E., *J. Am. Chem. Soc.* **1967**, *89*, 7009-7014.

[42] Schlosser, M., Christmann, K.F., *Angew. Chem. Int. Ed. Engl.* **1966**, *5*, 126.

[43] Schwab, J.M., Klassen, J.B., *J. Am. Chem. Soc.* **1984**, *106*, 7217-7227.

[44] Gerstein, J., Jencks, W.P., *J. Am. Chem. Soc.* **1964**, *86*, 4655-4663.

[45] Roblot, G., Wylde, R., *Tetrahedron* **1993**, *49*, 6381-6398.

[46] Schreiber, S.L., Meyers, H.V., Wiberg, K.B., *J. Am. Chem. Soc.* **1986**, *108*, 8274-8277.

[47] Marinier, A., Baettig, K., Dallaire, C., Pitteloud, R., Deslongchamps, P., *Can. J. Chem.*, **1989**, *67*, 1609-1617.

[48] Griesbaum, K., Jung, I.C., Mertens, H., *J. Org. Chem.* **1990**, *55*, 6024-6027.

[49] Kang, S-K., Kim, W-S., Moon, B-H., *Synthesis* **1985**, 1161-1162.

[50] Snider, B.B., Lu, Q., *J. Org. Chem.* **1996**, *61*, 2839-2844.

[51] Kulkarni, B.A., Chattopadhyay, S., Chattopadhyay, A., Mamdupar, V.R., *J. Org. Chem.* **1993**, 58 (22), 5964-5966.

[52] Tamura, M., Kochi, J., *Synthesis* **1971**, 303-305.

[53] Roush, W.R., Gillis, H.R., *J. Org. Chem.* **1982**, *47*, 4825-4829.

[54] Evans, D.A., Black, C.W., *J. Am. Chem. Soc.* **1993**, *115*, 4497-4513.

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